38th ANNUAL MEETING

Society for

INVERTEBRATE PATHOLOGY

PROGRAM and ABSTRACTS

7-11 August 2005

Anchorage,

Alaska

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Society for Invertebrate Pathology

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

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PROGRAM

2005

IMPORTANT NOTES:

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

STU indicates papers being judged for graduate student presentation awards

129 indicates abstract number for ORAL presentation

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

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

Opening Ceremonies and SIP Founders' Memorial Lecture

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

10:00–10:30	BREAK	BEB Lobby

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 Lo, Natl Taiwan Univ, Taiwan

11:00 2 **Molecular adaptations for pathogenicity in *Metarhizium anisopliae***. R St Leger, 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**. R ffrench-Constant, Univ of Bath, UK

12:30–2:00	LUNCH	Cuddy Center

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

Diseases of marine invertebrates

Organizer: Carolyn Friedman.

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

2:30 6 **Herpesviruses infecting bivalves**. T Renault, IFREMER Lab de Génétique, Aquaculture et Pathologie, La Tremblade, France

3:00 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 rickesttsial disease of abalone, *Haliotis* spp**. C Friedman, Univ of Washington, Seattle, WA, USA

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, DI Shapiro-Ilan**,** USDA, Agric Res Service, Byron, GA, USA

2:15 10 **Reduced susceptibility of over-wintering ladybirds to *Beauveria bassiana***. H Roy[1], E Ormond[1], M Majerus[2]. [1]Dept of Life Sciences, Anglia Polytechnic Univ, and [2]Dept 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[1], MH Griggs[2], E Groden[3], SL Annis[3], PK Mishra[3], JD Vandenberg[2]. [1]Dept of Entomology, Cornell Univ, [2]USDA-ARS, US Plant, Soil and Nutrition Lab, Ithaca, NY, [3]Dept 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[1], AL Gaitan[2], CE Gongora[1]. [1]Dept of Entomology, [2]Dept 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-hassanloui[1,2], A Kharazi-pakdel[1], MS Goettel[2], S Little[2], J Mozaffari [3]. [1]Dept of Plant Protection, College of Agriculture, Univ of Tehran, Karaj, Iran, [2]Lethbridge Research Centre, Lethbridge, Alberta, Canada, [3]Dept 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[1], M Keena[2], AE Hajek[1]**.** [1]Dept of Entomology, Cornell Univ, Ithaca, NY, USA, [2]USDA Forest Service, Northeastern Research Station Hamden, CT, USA

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

Contributed Papers Monday, 2:00-4:00. BEB 117

BACTERIA 1

Moderator: Alejandra Bravo.

2:00 17 **Characterization of the cadherin protein from *Lymantria dispar* as Cry1A toxin receptor.** JL Jurat-Fuentes[1], AP Valaitis[2], MJ Adang[1,3]. Depts of [1]Entomology and [3]Biochemistry & Molecular Biology, Univ of Georgia, Athens, GA, USA, [2]USDA For Serv, Delaware, OH, USA

2: 15 STU 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 Cyt1A.** SD Manceva[1], M Pusztai-Carey[2], P Butko[1]. [1]Dept of Chemistry and Biochemistry, Univ of Southern Mississippi, Hattiesburg, MS, [2]Dept of Biochemistry, Case Western Reserve University, Cleveland, OH, USA

2:45 **STU** **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 **STU** **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 STU 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 Brunet[1], V Vachon[1], M Marsolais[1], J van Rie[2], J-L Schwartz[1], R Laprade[1]. [1]Groupe d'étude des protéines membranaires, Univ de Montréal, Montréal, Québec and Biocontrol Network, Canada, [2]Bayer BioScience NV, Ghent, Belgium

3:30 STU 23 **Directed mutagenesis of conserved aromatic residues in helix 7 critical for larvicidal activity of the *Bacillus thuringiensis* Cry4Ba toxin.** K Tiewsiri, C Angsuthanasombat, Lab of Molec Biophys and Structl Biochem, Institute of Molecular Biology and Genetics, Mahidol Univ, Salaya Campus, Nakornpathom, Thailand

3:45 STU 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 Likitvivatanavong, C Angsuthanasombat. Lab of Molec Biophys and Structl Biochem, Institute of Molecular Biology and Genetics, Mahidol Univ, Salaya Campus, Nakornpathom, Thailand

4:00–4:20 BREAK BEB Lobby

Symposium (Div. of Microbial Control) Monday, 4:20-6:20. BEB 101

Use of pathogens against incursion pests

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

4:20 25 **Eradication of incursive lepidopteran pests with Foray.** R Fusco, A Rath. Valent 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 Hajek[1], JR Reilly[1], T Dubois[1], M Smith[2], L Bauer[3], Z Li[4]. [1]Dept Entomology, Cornell Univ NY, [2]USDA Newark, DE, [3]USDA Michigan, [4]Dept 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

Contributed Papers Monday, 4:20-6:20. BEB 117

VIRUSES 1

Moderator: Basil Arif.

4:20 30 **Pathogen diversity and the efficacy of virus insecticides**. JS Cory[1,2], DJ Hodgson[1,3], EM Redman[1]. [1]Ecology and Biocontrol Group, NERC Centre for Ecology and Hydrology, Oxford, UK, [2]Algoma Univ College, Sault Sainte Marie, Ontario, Canada, [3]School of Biological and Chemical Sciences, Univ of Exeter, Hatherly Laboratories, Devon, UK

4:35 STU 31 **The role of viral pathogens in the regulation of lepidopteran host populations: The winter moth and its natural enemies**. RI Graham[1], S Rao[2], SM. Sait[3], RD Possee[1], PPC Mertens[2], RS Hails[1]. [1]NERC Centre for Ecology and Hydrology, Oxford, UK, [2]Institute for Animal Health, Pirbright Laboratory, Woking, Surrey, UK, [3]Ecology and Evolution Research Group, School of Biology, Univ of Leeds, UK

4:50 STU 32 **Investigating the genetic parameters that affect virus transmission**. FL King[1], RS Hails[2], RD Possee[2], LA King[1]. [1]School of Molecular and Biological Sciences[1], Oxford Brookes Univ, Oxford, [2]NERC 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 Sayed[1,2], M Rezapanah[1,3], S Shojai-Estrabragh[1,4] JA Jehle[1]. [1]Lab of Biotechnological Crop Protection, Agricultural Service Center Palatinate, Neustadt/Wstr., Germany, [2]Dept of Economic Entomology and Pesticides, Faculty of Agriculture, Cairo Univ, Egypt, [3]Biocontrol Research Dept, Plant Pests and Diseases Research Institute, Tehran, Iran, [4]National Research Center of Genetic Engineering & Biotechnology, Tehran, Iran

5:20 STU 34 **Enhancement in activity of Turkish SpliNPV-B to *Spodoptera littoralis* Boisd. (Lepidoptera:Noctuidae) by an optical brightener**. U Toprak, O Gürkan, 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 Lee[1,2], JS Cory[3], K Wilson[2], D Raubenheimer[1], SJ Simpson[1]. [1]Dept of Zoology, Univ of Oxford, UK, [2]Dept of Biological Sciences, Institute of Environmental and Natural Sciences, Univ of Lancaster, UK, [3]Molecular 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 Grove[1], B Reed[1], AD Jones[1], N Hayes-Plazolles[2], J Slavicek[2], K Hoover[1]. [1]The Pennsylvania State Univ, Dept of Entomology, PA, USA, [2]USDA Forest Service, Delaware, OH, USA

6:05 37 **Effects of a protease-expressing recombinant baculovirus insecticide on the parasitoid *Cotesia marginiventris* (Cresson).** T Nusawardani[1], JR Ruberson[2], JJ Obrycki[3], BC Bonning[1]. [1]Dept of Entomology, Iowa State Univ, Ames, IA USA, [2]Dept of Entomology, Univ of Georgia, Tifton, GA USA, [3]Dept of Entomology, Univ of Kentucky, Lexington, KY, USA

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

MICROSPORIDIA AND PROTOZOA

Moderator: Andreas Linde.

4:20 38 **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 Pilarska[1], LF Solter[2], A Linde[3], M Kereselidze[4], G Hoch[5]. [1]Institute of Zoology, Bulgarian Academy of Sciences, Sofia, Bulgaria, [2]Center for Economic Entomology, Illinois Natl Hist Survey, Champaign, IL, USA [3]Dept of Forestry, Univ of Applied Sciences, Eberswalde, Germany, [4]V.Gulisashvili Institute of Mountain Forestry, Academy of Sciences, Tbilisi, Republic of Georgia, [5]Dept of Forest and Soil Sciences, BOKU Univ of Natural Resources and Applied Life Sciences, Vienna, Austria

4:35 39 **Effects of *Nosema fumiferanae* (Microspora) on distribution and dispersal of overwintering spruce budworm larvae**. K van Frankenhuyzen, C Nystrom**,** Great Lakes Forestry Centre, Canadian Forest Service, Sault Ste. Marie, Ontario, Canada

4:50 40 **Effect of microsporidia on the life history of the convergent lady beetle, *Hippodamia convergens* (Guérin-Méneville).** P Joudrey**,** Dept of Biology, Saint Mary's Univ, Halifax, Nova Scotia, Canada

5:05 41 **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 42 **Do *Johenrea locustae* and *Paranosema locustae* represent two different developmental sequences of the same species?** YY Sokolova[1,2], CE Lange[3], YS Tokarev[4], JJ Fuxa[1]. [1]Dept of Entomology, Louisiana State Univ AgCenter, Baton Rouge, Louisiana, USA, [2]Institute of Cytology, Russian Academy of Sciences, St. Petersburg, Russia, [3]Illinois Natl Hist Survey, Urbana IL, USA, and Center for Parasitological Studies, La Plata, Argentina, [4]Institute for Plant Protection, Russian Academy of Agricultural Sciences, St. Petersburg, Russia.

5:35 **43** **Microsporidian parasites in freshwater snails.**
STU HE McClymont[12], AM Dunn[1], RS Terry[1], D Rollinson[2], DTJ Littlewood[2], JE Smith[1]. [1]School of Biology, Univ of Leeds, UK, [2]The Natural History Museum, London, UK

5:50 44 **PfPuf2, a translational repressor, regulates sexual**
STU **development in the malaria parasite *Plasmodium falciparum*.** J Li[1], Q Fan[2], L Cui[1]. [1]Dept of Entomology, The Pensylvania State Univ, University Park, PA, USA, [2]Dept of BioScience and Technology, Dalian Univ of Technology, Liaoning, China

6:05 45 **Analysis of *Gregarines niphandrodes* mitochondria**.
STU MA Toso**,** CK Omoto, School of Biological Sciences, Washington State Univ, Pullman, WA

DINNER
6:20–8:00 Creekside Eatery – "dinner to go"
recommended for business meeting attendees

SIP Division Business Meetings: Monday evening

Viruses	(6:45-7:45p) Rm 107 HC
Microsporidia	(6:45-7:45p) Canary Rm, HC
Fungi	(6:45-7:30p) Rm 106 HC

Virus Division Workshop Monday, 7:45-8:45. Rm 107 HC

Microarray technology, genomics and proteomics in entomopathogen research

Organizers: James Maruniak and Peter Krell.

7:45 46 **Microarray technology, genomics and proteomics in entomopathogen research**. PJ Krell, Dept of Molecular and Cellular Biology, Univ of Guelph, ON, Canada

Additional featured speakers:
DA Theilmann, Agriculture and Agri-Food Canada, Pacific Agri-Food Centre, Summerland, BC, Canada; L Masson and M van Munster, Biotechnolgy Research Institute, NRC, Montreal, QC, Canada

Microsporidia Division Workshop,Monday, 7:45-8:45. Canary Rm HC

Transmission and ecology of Microsporidia: A broad spectrum of possibilities

Organizers: Regina G. Kleespies and Gernot Hoch.

7:45 47 **Experimental study of transmission of Microsporidia from blood-sucking mosquitoes of Siberia**. A Simakova, Federal State Unitary Enterprise of Ministry of Public Health of Russian Federation, Tomsk, Russia

8:00 48 **Epizootiology of a microsporidium in a blood-sucking mosquito population of Siberia**. T Pankova, Tomsk State Univ, Tomsk, Russia

8:15 49 **Exploring horizontal field transmission of Microsporidia**. V D'Amico[1], G Hoch[2], L Solter[3], [1]Univ of Delaware – USDA Forest Service, Newark, DE, USA, [2]BOKU-Univ of Natural Resources and Applied Life Sciences, Vienna, Austria, [3]Illinois Natl Hist Survey, Champaign, IL, USA

8:30 50 **Transmission of *Nosema fumiferanae* in spruce**
STU **budworm populations**. C Campbell, Univ of Toronto, Ontario, Canada

Fungus Division Workshop Monday, 7:30-9:30. Rm 106 HC

Systematics and ecology of Entomophthorales

Organizers: Siegfried Keller and Jørgen Eilenberg.

7:30 51 **Systematics of the arthropod-pathogenic Entomophthorales**. S Keller[1], R Humber[2], [1]FAL, Zürich, Switzerland, [2]USDA-Cornell, New York, USA

8:30 52 **Bizarre interactions and endgames: Entomopathogenic fungi and their arthropod hosts**. H Roy, Anglia Polytechnic Univ, Cambridge, UK

8:50 53 **Ecology of Entomophthorales: A European perspective**. J Eilenberg, The Royal Veterinary and Agricultural Univ, Frederiksberg, Denmark

9:10 54 **Ecological studies underpinning the development of conservation biological control with *Pandora neoaphidis* in UK**. JK Pell, Rothamsted Research, Harpenden, UK

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 Natl Hist Survey, Urbana, IL, USA

9:12 58 **Transmission of viruses to mosquito larvae mediated by divalent cations.** J Becnel, 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. Oi, 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***. E-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 ffrench-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 STU 67 **The characterisation of the structure of *Xenorhabdus* insecticidal toxin component XptA1.** SC Lee[1], S McPhie[2], A Rodger[3], DI Roper[2], J Henderson[5], M Sergeant[1], JAW Morgan[1]. [1]Warwick HRI, Univ of Warwick, Wellesbourne, Warwick, UK, [2]Dept of Biological Sciences, and [3]Dept of Chemistry, Univ of Warwick, Coventry, UK, [4]Univ of Nottingham, UK, [5]School of Biological Sciences, Coventry Univ, UK

9:00 STU 68 **The hemolysin alpha-xenorhabdolysin secreted by pathogenic enterobacteria belongs to a new family of cytotoxins and triggers apoptosis**. F Vigneux[1], A Givaudan[1], PA Girard[1], C Ribeiro[1], S Baghdiguian[2], M Brehélin[1]. [1]Laboratoire d'Ecologie Microbienne des Insectes Interactions Hôtes-Pathogènes, INRA-Univ de Montpellier II, France, [2]Institut 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 carpocapsae* (Nematoda: Steinernematidae).** SP Stock[1,2], Y Vega[1]. [1]Dept of Plant Sciences, [2]Dept of Entomology, University of Arizona. Tucson, USA

9:30 STU 70 **Genetic and molecular analysis of infective 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[1], RS Cowles[2], E Cowles[3], R Gaugler[4], AD Jones[5], DL Cox-Foster[1]**.** [1]Dept of Entomology, and [5]Dept of Chemistry, The Pennsylvania State Univ, PA, USA, [2]Valley Laboratory, The Connecticut Agricultural Experiment Station, Windsor, CT, USA, [3]Dept of Biology, Eastern Connecticut State Univ, Willimantic, CT, USA, [4]Dept of Entomology, The Rutgers Univ, New Brunswick, NJ, USA

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[1], R Becket[2], WA Miller[2], BC Bonning[1]. [1]Depts of Entomology and [2]Plant Pathology, Iowa State Univ, Ames, IA, USA

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

8:30 STU 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 Tentcheva, L Gauthier, B Dainat, F Cousserans, ME Colin, M Bergoin. 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, Unive of Florida, Gainesville, FL, USA

9:15 77 **Virus tropisms is controlled by insect parvovirus promoters**. P Tijssen, J Szelei, M El-Far, G Fédière, INRS-Institut Armand-Frappier, Laval, QC, Canada

9:30 STU 78 **Analysis of the immediate early *me53* gene from the baculovirus AcMNPV.** J de Jong[1], DA Theilmann[2], BM Arif[3], PJ Krell[1]. [1]Dept of Molecular and Cellular Biology, Univ of Guelph, ON, Canada, [2]Agriculture and Agri-food Canada, Pacific Agri-food Centre, Summerland, BC, Canada, [3]Canadian Forest Service, Great Lakes Forestry Research Centre, Sault Ste. Marie, ON, Canada

9:45 STU 79 **Characterization of *Cydia pomonella* 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 80 **A polydnavirus paradox: Cophylogeny and mosaic genomes**. J Whitfield, Univ of Illinois at Urbana-Champaign, IL, USA

10:50 81 **Polydnavirus genomics: Form and function of mutualistic insect viruses from parasitic wasps**. B Webb, Univ of Kentucky, Lexington, Kentucky, USA

11:20 82 **Inferring evolution through the biology of ascoviruses.** X-W Cheng, Miami Univ, Oxford, Ohio, USA

11:50 83 **The biology of polydnaviruses and their interactions with insect hosts**. N Beckage, Univ of California, Riverside, CA, USA

Contributed Papers Tuesday, 10:20-11.35. BEB 110

ALGAE, OTHER

Moderator: James Becnel.

10:20 84 **Women pioneers of invertebrate cell culture**, Karl Maramorosch, Dept of Entomology, Rutgers Univ, New Brunswick, New Jersey, USA

10:35 85 **Genomics approaches to insect-pathogen relationships in the spruce budworm, *Choristoneura fumiferana***. Q Feng[1], T Ladd[1], S Zheng[1, 2], L Li[1], D Zhang[1,2], D Buhlers[1], PJ Krell[2], BM Arif[1], A Retnakaran[1]. [1]Great Lakes Forestry Centre, Canadian Forest Service, Sault Ste. Marie, Ontario, Canada, [2]Dept of Microbiology, Univ of Guelph, ON, Canada

10:50 86 **Development and pathway of infection of the entomopathogenic alga *Helicosporidium* (Chlorophyta: Trebouxiophyceae).** V-U Bläske-Lietze, DG Boucias, Entomology and Nematology Dept, Univ of Florida, Gainesville, FL, USA

11:05 STU **87** ***Helicosporidium* sp. infection in mosquito larvae**. TM Conklin[1], V-U Bläske[1], JJ Becnel[2], DG Boucias[1]. [1]Dept of Entomology and Nematology, Univ of Florida, Gainesville FL, USA, [2]USDA, CMAVE, Gainesville, FL, USA

11:20 STU 88 **Identification of genes transcribed by *Moraxella osloensis* in slug *Deroceras reticulatum* using selective capture of transcribed sequences**. R An, S Sreevatsan, P Grewal, Dept of Entomology, OARDC, The Ohio State Univ, OH, USA

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

POSTERS – 1

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 Nam[1], I-P Hong[1], J-S Hwang[1], S-B Hong[2], S-D Ji [1], S-W Kang[1], M-S Han [3]. [1]Dept of Agricultural Biology, and [2]National Institute of Agricultural Biotechnology, National Institute of Agricultural Science Technology, RDA, Suwon, Korea, [3]College of Agriculture and Life Science, 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-hassanloui[1], A Kharaz-pakdel[1], MS Goettel[2], J Mozaffari[3], J Bissett[4]. [1]Dept of Plant Protection , College of Agriculture, Univ of Tehran, Karaj, Iran, [2]Lethbridge Research Centre, Lethbridge, Alberta, Canada, [3]Dept of Genetics, Seed and Plant Improvement Institute, Karaj, Iran, and [4]ECORC, Ottawa, Ontario, Canada

F-4 **Approaches to testing a biological hypothesis that host flight dominates transmission of aphid-pathogenic fungi among aphid populations.** C Chen[1], M-G Feng[1, 2]. [1]Institute of Microbiology, College of Life Sciences, [2]Institute 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. Salazar[1], V. Cañedo[1], J. Alcázar[1], A. Lagnaoui[2]. [1]International Potato Center (CIP), Lima, Peru, [2]The World Bank, Environmentally and Socially Sustainable Development, Washington DC, USA

F-7 STU **Factors relating to epizootics of *Hirsutella* sp. in field populations of *Homalodisca coagulata* (Say) (Homoptera: Cicadellidae)**. SE Breaux[1], DG Boucias[1], RF Mizell III[2], [1]Univ of Florida, Dept of Entomology and Nematology, Gainesville, FL, USA, [2]Univ of Florida, North Florida Research and Education Center Quincy, FL, USA

F-8 **Growth and virulent characteristics of *Verticillium lecanii* (*Lecanicillium* spp.) hybrid strains.** D Aiuchi[1], M Koike[1], K Inami[1], Y Baba[1], M Sugimoto[2]. [1]Dept of Agro-environmental Science, Obihiro Univ of Agriculture and Veterinary Medicine, Hokkaido, Japan, [2]Okinawa Prefectural Agricultural Experiment Station, Naha, Japan

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

F-10 STU **Interactions between over-wintering seven spot ladybirds (*Coccinella septempunctata*) and the entomopathogenic fungus *Beauveria bassiana*: The 12 buckets**. E Ormond[1], A Thomas[1], J Pell[2], H Roy[1]. [1]Dept of Life Sciences, Anglia Polytechnic Univ, Cambridge, UK,

[2]Plant and Invertebrate Ecology Division, Rothamsted Research, Harpenden, UK

F-11 **Comparison of *Galleria* baiting and soil plating methods for isolating soilborne pathogens from the habitats of glassy-winged sharpshooter, *Homalodisca coagulata* (Homoptera: Cicadellidae), in California.** S K Dara[1], MR McGuire[2], HK Kaya[3]. [1]Shafter Research and Extension Center, Shafter, CA, [2]USDA-ARS, Shafter, CA, [3]Dept 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 Bignayan[1,2], DEN Rangel[1], EW Evans[1], DW Roberts[1]. [1]Dept of Biology, Utah State Univ, Logan, UT, USA, [2]Bureau of Plant Industry, National Mango Research and Dev Center, Jordan, Guimaras, Philippines

F-14 **Isolates of *Metarhizium anisopliae* are diverse in their relationships between pigments and stress tolerance**. DEN Rangel[1], GUL Braga[1,2], AJ Anderson[1], DW Roberts[1]. [1]Dept of Biology, Utah State Univ, Logan, UT, USA, [2]Dept de Análises Clínicas, Toxicológicas e Bromatológicas, Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Univ de São Paulo, Ribeirão Preto, Brazil

F-15 STU **Are 'stressed-out' wireworms more susceptible to the biocontrol agent *Metarhizium anisopliae*?** J Ericsson[1,2], JT Kabaluk[2], M Goettel[3], M Isman[1], E Jovel[1], JH Myers[1]. [1]Faculty of Agricultural Sciences, Univ of British Columbia, Vancouver, BC, and Agriculture and Agri-Food Canada, [2]Pacific Agricultural Research Centre, Agassiz, BC, Canada, [3]Lethbridge Research Centre, Lethbridge, Alberta, Canada

F-16 **Challenges and constraints in deploying *Metarhizium anisopliae* for biocontrol of sugarbeet root maggot, *Tetanops myopaeformis*.** ST Jaronski, JA Grace, R Schlotauer, USDA ARS NPARL, Sidney MT, USA

F-17 **Observations on the interaction between biocontrol fungi, *Metarhizium* and *Beauveria*, and bacteria isolated from the rhizosphere of sugar beets.** K Jung[1], C Fuller-Schaefer[2], B Larson[3], ST Jaronski[2]. [1]Federal Biological Research Centre for Agriculture and Forestry, Institute for Biological Control, Darmstadt, Germany, [2]USDA-ARS Northern Plains Agricultural Research Laboratory, Sidney MT, USA, [3]Richland County MT Extension Service, Sidney MT, USA

F-18 **Influence of plant rhizosphere on the abundance of entomopathogenic fungi.** DR Sosa-Gómez, AMR Almeida, JJ da Silva, LC Benato, Embrapa Soja, Brazil

F-19 **Colonization of sugarbeet roots by entomopathogenic fungi**. C Fuller-Schaefer[1], K Jung[2], S Jaronski[1]. [1]USDA-ARS, Northern Plains Agricultural Research Lab, Sidney, MT, USA, [2]Federal Biological Research Center for Agriculture and Forestry, Institute for Biological Control, Darmstadt, Germany

F-20 **Coffee endophytes pathogenic to the coffee berry borer.** F Posada, FE Vega, Insect Biocontrol Laboratory, USDA-ARS, Beltsville, Maryland

F-21 **Low likelihood of recombination between the introduced *Beauveria bassiana* strain GHA and indigenous conspecific strains based on vegetative compatibility groupings**. LA Castrillo[1], SL Annis [2], E Groden[2], PK Mishra[2], JD Vandenberg[3], [1]Dept of Entomology, Cornell Univ, Ithaca, New York, [2] Dept of Biological Sciences, Univ of Maine, Orono, ME, [3] USDA-ARS, US Plant, Soil and Nutrition Lab, Tower Road, Ithaca, NY, USA

F-22 **Purification and gene cloning of a new hydrophobin-like protein that relates to thermal tolerance of aerial conidia of fungal biocontrol agents.** S-H Ying[1], M-G Feng[1, 2]. [1]Institute of Microbiology, Coll of Life Sciences, and [2]Institute of Applied Entomology, Coll of Agriculture and Biotechnology, Zhejiang Univ, Hangzhou, Zhejiang, PR China

F-23 **Toxins are overproduced in a gene disruption mutant of *Metarhizium anisopliae***. SB Krasnoff[1], Y-S Moon[2], BGG Donzelli[1,2], ACL Churchill[1,2], JD Vandenberg[3], DM Gibson[3], [1]Dept of Plant Pathology, Cornell Univ, Ithaca, NY, [2]Boyce Thompson Institute, Ithaca, NY, [3]USDA-ARS, Plant Protection Research Unit, Ithaca, NY, USA

F-24 STU **A study of the expression profile of pathogenicity related genes in the entomopathogenic fungus *Beauveria bassiana* on different insect cuticles.** PAA Khan[1], KU Devi[1], A Reineke[2]. [1]Dept of Botany, Andhra Univ, Visakhapatnam, India, [2]Dept of Entomology, Max-Planc Institute of Chemical Ecology, Jena, Germany

F-25 **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-26 **Targeted disruption of a peptide synthetase gene in *Metarhizium anisopliae* has no effect on destruxins production or virulence against insects**. Y-S Moon[1], SB Krasnoff[2], BGG Donzelli[1,2], JD Vandenberg[3], DM Gibson[3], ACL Churchill[1,2]. [1]Boyce Thompson Institute, Ithaca, NY, [2]Dept of Plant Pathology, Cornell Univ, Ithaca, NY, [3]USDA-ARS, Plant Protection Research Unit, Ithaca, NY, USA

BACTERIA

B-1 **Vip3Ba1: A novel Vip protein from *Bacillus thuringiensis***. C Rang[1], P Gil[1], N Neisner[1], J van Rie[1], R Frutos[2]. [1]Bayer BioScience NV, Gent, Belgium, [2]CIRAD, Campus International de Baillarguet, Montpellier, France

B-2 STU **Identification of *vip* genes in *Bacillus thuringiensis* strains by PCR-RFLP**. CS Hernández[1], A Boets[2], J van Rie[2], J Ferré[1]. [1]Departament de Genètica, Univ de València, Spain, [2]Bayer 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, LI Burtseva, Lab of Insect Pathology, Institute of Animal Systematics and Ecology, SB RAS, Novosibirsk, Russia

B-5 **Molecular cloning of novel crystal protein genes, *cry30C* and *s2orf2*, from a mosquitocidal *Bacillus thuringiensis* serovar *sotto* strain**. A Ohgushi[1], N Wasano[2], H Saitoh[2], A Uemori[1], M Ohba[1]. [1]Graduate School of Agriculture, Kyushu Univ, Fukuoka, Japan, [2]Biotechnology and Food Research Institute, Kurume, Fukuoka, Japan

B-6 **Utilisation of the Rhs core region of *tc-sepC* orthologes 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 Bouillaut[1], C Buisson[1], N Ramarao[1], M Gohar[1], D Lereclus[1], C Nielsen-LeRoux[1,2].

[1]Unité Génétique Mirobienne et Environnement, INRA, la Minière, France, [2]Dépt Microbiologie Fondamentale et Médicale, Institut Pasteur, Paris, France

B-8 STU **_Xenorhabdus nematophila_ secreted proteases and their role in insect pathogenesis.** C Lipke, H Goodrich-Blair**,** Univ of Wisconsin-Madison, Madison, Wisconsin, USA

B-9 **An elastase found in early instar _Lymantria dispar_ larvae is involved in the proteolytic activation of _Bacillus thuringiensis_ toxins**. AP Valaitis, USDA Forest Service, Delaware, OH, USA

B-10 **Influence of bacteriocin metabolites of _Bacillus thuringiensis_ on antioxidants of gut of _Galleria mellonella_ larvae**. EL Dzju, NV Redkina, VV Glupov, Lab of Insect Pathology, Institute of Systematic and Ecology of Animals, Russian Academy of Sciences, Novovsibirsk, Russia

B-11 **Functional analysis of the cadherin protein from _Heliothis virescens_ as Cry1Ac receptor**. JL Jurat-Fuentes[1], MJ Adang[1,2], Depts of [1]Entomology and Biochemistry & Molecular Biology[2], Univ of Georgia, Athens, GA, USA

B-12 **CR12-MPED fragment of _Manduca sexta_ Bt-R_{1a} cadherin enhances activity of Bt Cry1A toxins**. G Hua[1], J Chen[1], JL Jurat-Fuentes[1], MA Abdullah[1], M Adang[1,2]. Depts of Entomology[1] and Biochemistry & Molecular Biology[2], Univ of Georgia, Athens, GA, USA

B-13 **Mutagenic analysis of surface-exposed loop residues critical for larvicidal activity of the _Bacillus thuringiensis_ Cry4Ba toxin**. T Khaokhiew, C Angsuthanasombat, 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 Leetachewa, C Angsuthanasombat, Lab of Molecular Biophysics and Structural Biochemistry, Institute of Molecular Biology and Genetics, Mahidol Univ, Nakorn-Pathom, Thailand

B-15 STU **Isolation and functional characterization of _Bacillus thuringiensis_ Cry4Ba toxin-binding proteins from _Aedes aegypti_ larvae**. S Moonsom, C Angsuthanasombat, Lab of Molecular Biophysics and Structural Biochemistry, Institute of Molecular Biology and Genetics, Mahidol Univ, Nakorn-Pathom, Thailand

B-16 **Interaction of the Bt toxin Cyt1A with lipid monolayer**. S Clark[1], M Pusztai-Carey[2], P Butko[1]**.** [1]Dept of Chemistry and Biochemistry, Univ of Southern Mississippi, Hattiesburg, MS, [2]Dept of Biochemistry, Case Western Reserve Univ, Cleveland, OH, USA

B-17 STU **Exposing the cryptic antibacterial potential of Cyt1Ca from _Bacillus thuringiensis israelensis_ by genetic manipulations**. M Itsko[1], R Manasherob[2], C Berry[3], A Zaritsky[1]. [1]Dept of Life Sciences, Ben-Gurion Univ of the Negev, Be'er-Sheva, Israel, [2]Dept of Genetics, Stanford Univ, Stanford, CA, USA, [3]Cardiff School of Biosciences, Cardiff Univ, Cardiff, UK

B-18 **Endogenic activation of Cyt2Ba toxin by camelysin from _Bacillus thuringiensis israelensis_.** M Nisnevitch[1], S Cohen[1,2], E Ben-Dov[2] , A Zaritsky[2], R Cahan[1]. [1]Dept of Chemical Engineering and Biotechnology, College of Judea and Samaria, Ariel, Israel, [2]Dept of Life Sciences, Ben-Gurion Univ of the Negev, Be'er-Sheva, Israel

B-19 **Individual characterization of the three _cyt1A_ promoters of _Bacillus thuringiensis_ subsp. _israelensis_**. Y Sakano[1], H-W Park[2], BA Federici[1]. [1]Dept of Entomology, Univ of California, Riverside, CA, USA, [2]John 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. _tenebrionis_**. 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 Jara[1], P Maduell[1,2], S Orduz[1,3]. [1]Unidad de Control Biológico y Biotecnología, Corporación para Investigaciones Biológicas, Medellín, Colombia, [2]Unitat de Microbilogia, Facultat de Ciencias, Univ Autonoma de Barcelona, Barcelona, España, [3]Facultad de Ciencias Basicas, Univ de Pamplona, Pamplona, Colombia

B-23 **Characterization of selected _Bacillus thuringiensis_ strains.** GV Kalmykova[1], LI Burtseva[1], AV Mokeeva[2], SF Oreshkova[2]. [1]Lab of Insect Pathology, Institute of Animal Systematics and Ecology, SB RAS, Novosibirsk, Russia, [2]Vector State Research Center of Virology and Biotechnology, Institute of Bioengineering, Kol'tsovo, Novosibirskaya oblast, Russia

B-24 **Effects of _Bacillus thuringiensis_ var. _kurstaki_ toxins on _Scheloribates praeincisus_ (Berlese, 1910) (Acari: Oribatida: Scheloribatidae).** AR Oliveira, I Delalibera Jr, TR Castro**,** Dept of Entomology, Plant Pathology and Agricultural Zoology, ESALQ – Univ of São Paulo, Brazil

B-25 **Effect of _Bacillus thuringiensis_ strains on _Spodoptera cosmioides_**. PJ Neves[1], KB Santos[1], AM Meneguim[2], GT Vilas-Bôas[1], WJ Santos[2], OMN Arantes[1]. [1]Univ Estadual de Londrina-UEL, Londrina-PR, Brazil, [2]Instituto Agronômico do Paraná-IAPAR, Brazil

B-26 **Study on preparation of _Bacillus thuringiensis_ controlling both Lepidopetera and Coleoptera pest**. P Cheng[1] , M Sun[2], Z Yu[2], S Chen[2], H Xie[1], G Yu[1]. [1]Zhuhai Agricultural Science Research Centre, Zhuhai, Guangdong, PR China, [2]Dept 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 Taveecharoenkool[1], T Kerdcharoen[2], C Angsuthanasombat[3]. [1]Dept of Immunology, Siriraj Hospital, and [2]Dept of Physics and Capability Building Center for Nanoscience and Nanotechnology, Mahidol Univ, Bangkok, Thailand, [3]Lab 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, _Cyrtomenus bergi_ Froeschner (Hemiptera: Cydnidae)**. 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 concordis_ (Acari: Phytoseiidae)**. FH Ibanhes, I Delalibera Jr**,** Dept of Entomology, Plant Pathology and Agricultural Zoology, ESALQ – Univ of São 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-Simón[1], C Avilla[2], JE González-Zamora[2], J Ferré[1]. [1]Departamento de Genética, Univ de Valencia. Valencia, Spain, [2]Departamento de Ciencias Agroforestales, Univ de Sevilla, Spain

B-31 **Treatment of an *Aedes aegypti* colony during 33 generations with the Cry11Aa toxin of *Bacillus thuringiensis* serovar. *israelensis* results in moderate resistance development**. OE Guevara[1], A Builes[1], S Orduz[1,2]. [1]Unidad de Biotecnología y Control Biológico, Corporación para Investigaciones Biológicas (CIB), Medellín, Colombia, [2]Facultad de Ciencias Básicas, Univ de Pamplona, Colombia

B-32 **Biology and nutrition of resistant and susceptible populations of *Anticarsia gemmatalis* (Lepidoptera: Noctuidae) to *Bacillus thuringiensis*.** F Moscardi [1], AB Malaguido[2], CB Hoffmann Campo[1]. [1]Embrapa Soja, [2]Embrapa-Soja/Pronex, Londrina-PR, Brazil

B-33 STU **A common, but complex, mode of resistance of *Plutella xylostella* to *Bacillus thuringiensis* toxins Cry1Ab and Cry1Ac**. S Ibiza-Palacios[1], AH Sayyed[2], R Gatsi[2], DJ Wright[2], N Crickmore[3], B Escriche[1]. [1]Dept de Genetica, Univ de Valencia, Spain, [2]Division of Biology, Imperial Coll London, Ascot, Berkshire UK, [3]School of Life Sciences, 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-Simón[1], P Wang[2], J Zhao[2], A Shelton[2], J Ferré[1]. Dept de Genética, Univ de Valencia, Spain, [2]Dept 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-Cabrera[1], HA Siqueira[2], BD.Siegfried[2], J Ferré[1]. [1]Dept de Genética, Facultad de CC. Biológicas, Univ de Valencia, Spain, [2]Dept of Entomology, Univ of Nebraska-Lincoln, NE, USA

B-36 **Reduction in levels of the *Heliothis virescens* alkaline phosphatase (HvALP) as a marker for resistance to Cry1Ac.** JL Jurat-Fuentes[1], MJ Adang[1,2]. Depts of [1]Entomology and Biochemistry & Molecular Biology[2], Univ of Georgia, Athens, GA 30602, USA

B-37 **Could insect gut esterases be a threat to Bt crops?** AH Sayyed[1], MJ Bruce[1], DJ Wright [2], N Crickmore [1]. [1]Dept of Biochemistry, Univ of Sussex, Brighton UK, [2]Division of Biology, Imperial College London, Ascot, Berks, UK

B-38 STU **Analysis of midgut proteinases from *Bacillus thuringiensis* susceptible and resistant to *Heliothis virescens* (Lepidoptera: Noctuidae).** L Karumbaiah[1], B Oppert[3], JL Jurat-Fuentes[1], MJ Adang[1,2]. Depts of Entomology[1], Biochemistry and Molecular Biology[2], Univ of Georgia, Athens, GA, [3]USDA ARS Grain Marketing and Production Research Center[3], 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 Gringorten[1], Y Choi[2], L Morel[2], L Masson[2], C Miguez[2]. [1]Great Lakes Forestry Centre, Canadian Forest Service, Sault Ste. Marie, Ontario, Canada, [2]Biotechnology Research Institute, National Research Council of Canada, Montreal, Quebec Canada

EXCURSION:

12:30-5:30 Discover Alaska Tour (lunch included)
Meet bus at North Parking Lot near Cuddy Center
Buses leave at 12:30 prompt

6:30-7:15 Buses leave for BBQ

7:00-12:00 Alaska Native Heritage Center BBQ

10:00-12:00 Buses return from BBQ

WEDNESDAY - 10 August

Symposium (Nematode Division) Wednesday, 8:00–10:00. BEB 101

Genomics of entomopathogenic nematodes and symbiotic bacteria

Organizer/Moderator: Parwinder Grewal.

8:00 89 **The *Xenorhabdus* genome project**. S Forst, Univ of Wisconsin, Milwaukee, WI, USA

8:20 90 ***Photorhabdus*: Functional genomics of an insect pathogen**. R ffrench-Constant, Univ of Bath, Bath, UK

8:40 91 **Nutrition and signal exchange between *Photorhabdus* and its invertebrate hosts**. D Clark, Univ of Bath, Bath, UK

9:00 92 ***Heterorhabditis bacteriophora* genome project: A glimpse into the first 1000 expressed sequence tags**. P Grewal, Ohio State Univ, Wooster, Ohio, USA

9:20 93 **Developing tools for genetics and genomics in *Heterorhabditis bacteriophora***. A Fodor, Ohio State Univ, Wooster, Ohio USA

9:40 94 **Application of forward and reverse genetics for the study of symbiosis in an entomopathogenic nematode host**. T Ciche, Michigan State Univ, East Lansing, MI, USA

Contributed Papers Wednesday, 8:00-9:45. BEB 110

FUNGI 2

Moderator: John Vandenberg and Siegfried Keller.

8:00 96 **A molecular diagnostic method for selected *Ascosphaera* species using PCR amplification of internal transcribed spacer regions of rDNA.** KD Murray[1], KA Aronstein[2], WA Jones[3]. [1]Texas A&M Agricultural Experiment Station, Weslaco, TX, [2]USDA-ARS, Honey Bee Research Unit, Kika de la Garza Subtropical Agricultural Center, Weslaco, TX, [3]USDA-ARS, Beneficial Insects Research Unit, Weslaco, TX, USA

8:15 STU 97 **Multiple genetic lineages coexist sympatrically within a local population of *Beauveria bassiana* s.l.** NV Meyling[1], SA Rehner[2], M Lübeck[3], EP Buckley[2], J Eilenberg[1]. [1]Dept of Ecology, and [3]Dept of Plant Biology, Royal Veterinary and Agricultural Univ, Frederiksberg C, Denmark, [2]Insect Biocontrol Laboratory, USDA-ARS, Beltsville, MD, USA

8:30 98 **Characterization of a *Beauveria bassiana* isolate from feral black-legged ticks, *Ixodes scapularis* (Say)**. LB Flor, TJ Kurtti, UG Munderloh, Dept of Entomology, Univ of Minnesota, St. Paul, MN, USA

8:45 STU 99 **The role of oxalic acid in the pathogenicity of *Beauveria bassiana* towards Ixodidae tick species**. B Kirkland, NO Keyhani, Univ of Florida, Microbiology and Cell Science, Gainesville FL, USA

9:00 100 **Iron acquisition in the entomopathogenic fungus** ***Beauveria bassiana***. G Westwood, NO Keyhani**,** Univ of Florida, Microbiology and Cell Science, Gainesville, FL, USA
STU

9:15 101 **Comparison of different methods for isolation of entomopathogenic fungi from soil.** W Mandefro[1], M Dawd[1], S Gouli[2]. [1]Ethiopian Agricultural Research Org, Ambo, Ethiopia, [2]Entomology Research Lab, Univ of Vermont, Burlington, USA

9:30 102 **Virulence and sporulation of *Metarhizium anisopliae* in the presence of *Trichoderma conidia* on agar substrates and in soil bioassays on larvae of the black vine weevil.** L Hjeljord[1], R Meadow[2]. [1]Norwegian Univ of Life Sciences, Aas, Norway, [2]The Norwegian Crop Research Institute, Aas, Norway

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[1], JA Kroemer[2], BA Webb[2], N Chejanovsky[1]. [1]Entomology Dept, Institute of Plant Protection, The Volcani Center, Bet Dagan, Israel, [2]Dept of Entomology, Univ of Kentucky, Lexington, KY, USA

8:15 104 **Hemocyte variations relating to age related immunocompetancy in gypsy moth (*Lymantria dispar*).** J McNeil, D Cox-Foster, M Grove, K Hoover, Dept of Entomology, Pennsylvania State Univ, University Park, PA, USA
STU

8:30 105 **Molecular cloning of *Choristoneura fumiferana* prophenoloxidases 1 and 2 and their regulation by a polydnavirus.** D Doucet[1], Q Feng[2], M Cusson[1]. [1]Laurentian Forestry Centre, Natural Resources Canada, Quebec, Canada, [2]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 Dumpit[1], NE Beckage[2]. [1]Dept of Biochemistry and Molecular Biology, and [2]Depts 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, Umea Centre for Molecular Pathogenesis, Umea 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

9:30 109 **Transcriptome studies on the penaeid shrimp biodefense-related genes.** T Aoki[1], I Hirono[1], M Yasuike[1], K Sangrungrunang[2], R Ueno[3], L Ruangpan[4], Y Takahashi[5], R Wongpanya[6], A Tassanakajon[6]. [1]Tokyo Univ of Marine Science and Technology, Japan, [2]Kung Krabaen Bay Fisheries Development Study Centre, Thailand, [3]Mie Univ, Japan, [4]Chanthaburi Coastal Fisheries Research and Development Center, Thailand, [5]National Fisheries Univ, Japan, [6]Chulalongkorn Univ, Thailand

9:45 110 **Characterization and expression analysis of biodefense-related genes from kuruma shrimp, *Marsupenaeus japonicus*.** I Hirono, T Aoki. Lab of Genome Science, Tokyo Univ of Marine Science and Technology, Minato, Japan

10:00–10:30 BREAK BEB Lobby

Symposium (Cross-Div.) Wednesday, 10:30–12:30. BEB 101

Invertebrate responses to pathogens

Organizers/Moderators: Christina Nielsen-LeRoux and James Maruniak.

10:30 111 ***B. thuringiensis*, pore-forming toxins, and their interactions with *C. elegans*.** R Aroian, Section of Cell and Developmental Biology, Univ of California, San Diego, USA

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.** S Herrero, Plant Research International BV, and Lab of Virology, Wageningen Univ, The Netherlands

11:30 113 **Infection and cell-specific replication of the most successful viral insecticide, *Anticarsia gemmatalis* nucleopolyhedrovirus**. B Riberio, Instituto de Ciencias Biologias, Univ de Brasilia, Brazil

12:00 114 **Immune depression triggered in insects by the bacteria *Xenorhabdus nematophila* and *Photorhabdus lumniscens*.** M Brehelin, INRA and Univ of Montpellier, France

Contributed Papers Wednesday, 10:30-12:30. BEB 111

NEMATODES

Moderator: Guy Belair.

10:30 115 **Do entomopathogenic nematodes have potential as biological control agents of stored product insects?** JF Campbell[1], O Ramos-Rodriguez[2], S Ramaswamy[2].[1]USDA ARS, and [2]Dept. Entomology, Kansas State Univ, Manhattan, Kansas, USA

10:45 116 **Control of navel orangeworm in fallen pistachios using large scale application of the entomopathogenic nematode, *Steinernema carpocapsae*.** JP Siegel[1], LA Lacey[2], P Noble[1], J Bettiga[3], B Higbee[4], R Fritts, Jr[5]. [1]USDA/ARS, SJVASC, Parlier, CA,[2]USDA/ARS, YARL, Wapato, [3]S & J Ranch, Madera, CA, [4]Paramount Farming Company, Bakersfield, CA,[5]Certis USA, Columbia, MD, USA

11:00 117 **Laboratory characterization of *Steinernema carpocapsae, S. feltiae*, and *Heterorhabditis bacteriophora* for a multi-species biological control approach targeting the alfalfa snout beetle, *Otiorhynchus ligustici* (L.).** G Neumann, E Shields, Cornell Univ, USA
STU

11:15 118 **Improvement of *Steinernema carpocapsae* for control of the pecan weevil, *Curculio caryae* through hybridization and bacterial transfer.** DI Shapiro-Ilan,[1] RJ Stuart[2], CW McCoy[2]. [1]USDA-ARS, Southeast Fruit and Tree Nut Research Lab, Byron, GA, [2]Citrus Research and Education Center, IFAS, Univ of Florida, Lake Alfred, FL, USA

11:30 119 **Evaluating efficacy of application of entomopathogenic nematodes in drip-line irrigation systems.** AP Brown[1], JD Pearce[2], DJ Wright[1]. [1]Division of Biology, Imperial College London, Ascot, Berkshire, UK, [2]BeckerUnderwood, Littlehampton, W Sussex, UK
STU

11:45 120 **Soil biology of entomopathogenic nematode, *Steinernema abbasi* and the impacts of host plants on its pathogenicity.** W-F Hsiao, H-J Yu, Graduate Institute of Bioresources, Natl Chiayi Univ, Chiayi, Taiwan

12:00 **STU** 121 **Infection preferences of an entomopathogenic nematode, *Steinernema riobrave*.** JM Christen[1], JF Campbell[2], SB Ramaswamy[1]. [1] Kansas State Univ, Dept of Entomology, and [2]USDA-ARS, Grain Marketing and Production Research Center, Manhattan, KS, USA

12:15 122 **Movement, colonization, and persistence of the entomopathogenic nematode *Heterorhabditis marelatus* in a California coastal grassland.** DS Gruner[1], DR Strong[1,2], K Ram[2]. [1]Bodega Marine Lab, and [2]Section of Ecology and Evolution, Univ of California, Davis, USA

Contributed Papers Wednesday, 10:30-12:30. BEB 117

MICROBIAL CONTROL 1

Moderator: Wendy Gelernter.

10:30 123 **Sphaerus® SC, a Brazilian bioinsecticide to control the vector of malaria and filariases.** R Monnerat[1], CM Soares[2], [1]Embrapa Recursos Genéticos e Biotecnologia, and [2]Bthek Biotecnologia Ltda, Brasília, Brazil

10:45 124 **Bt-horus® SC, a Brazilian bioinsecticide to control mosquitoes and black-flies.** R Monnerat[1], CM Soares[2], [1]Embrapa Recursos Genéticos e Biotecnologia, and [2]Bthek Biotecnologia Ltda, Brasília, 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 Fabrick, MS Sisterson, S Morin, TJ Dennehy, Y Carrière, BE Tabashnik, Dept of Entomology, Univ of Arizona, Tucson, USA

11:30 127 **Evaluation of two formulations based on microbial metabolites to the control of blackcurrant insect pests.** MV Shternshis[1], MA Vaskin[1], VV Gouli[2]. [1]Novosibirsk State Agrarian Univ, Russia, [2]Univ 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 Frankenhuyzen, Great Lakes Forestry Centre, Canadian Forest Service, Sault Ste. Marie, Ontario, Canada

12:00 **STU** 129 **Cloning and expression of cry1Ah1 gene from isolate of *Bacillus thuringiensis* and its bioactivity.** H Li[1,2], J Tan[1], L Han[2], K He[1], G Liang[1], F Song[1], D Huang[3], J Zhang[1]. [1]State Key Lab for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Acad of Agricultural Sciences, Beijing, [2]College of Life Sciences, Northeast Agric Univ, Harbin, [3]Biotechnology Research Institute, CAAS, Beijing, PR China

12:15 **STU** 130 **Characterization of a *Bacillus thuringiensis* strain Bt185 toxic to the Asian cockchafer: *Holotrichia parallela*.** H Yu[12], F Song [1], J Zhang[1], J Gao[2]. [1]State Key Lab of Biology for Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agric Sciences, Beijing, [2]Northeast Agricultural Univ, HarBin, PR China

12:30–2:00 LUNCH Cuddy Center

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

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

Contributed Papers Wednesday, 2:00-4:00. BEB 101

BACTERIA 2

Moderator: Jeroen van Rie.

2:00 135 **Quantification of the dose of lepidopteran activity in new cotton events expressing the insecticidal protein Vip3A.** D O'Reilly, N Dupen, J Cairns, K Windle, R Hughes, M Gill, A Blake, J Sheridan, Syngenta, Jealotts Hill Research Center, Bracknell, Berks, UK

2:15 **STU** 136 **Identification of vip3A-type genes from *Bacillus thuringiensis* strains and characterization of two novel vip3A-type genes.** J Liu[1,2], F Song[1], J Zhang[1], J Tan[2]. [1]State Key Lab of Biology for Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, [2]Agricultural Univ of HeBei, PR China

2:30 137 **Two novel classes of secreted insecticidal proteins of *Bacillus thuringiensis*.** WP Donovan[1], JT Engleman[2], JC Donovan[2], WP Clinton[1], OM Ilagan[1], KC Krasomil-Osterfeld[1], TM Malvar[1], JW Pitkin[1], MR Walters[1], JA Baum[1], JK Roberts[1]. [1]Monsanto Company, St. Louis, MO, [2]Ecogen Incorporated, Langhorne, PA, USA

2:45 138 **Enterotoxigenic genes: Are they involved in insecticidal activity in *Bacillus thuringiensis*?** G Kyei-Poku, D Gauthier, K van Frankenhuyzen, Great Lakes Forestry Centre, Canadian Forest Service, Sault Ste. Marie, Ontario, Canada

3:00 **STU** 139 **Cloning and mutation of the cry8C-type gene from *Bacillus thuringiensis* toxic *Anomala corpulenta*.** C Shu[1], F Song[1], S Feng[2], R Wang[2], J Zhang[1]. [1]State Key Lab of Biology for Plant Diseases and Insect Pests, Institute of Plant Protection, Beijing, [2]Institute of Plant Protection, HeBei Academy of Agricultural and Forest Sciences, PR China

3:15 **STU** 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

3:30 **STU** 141 **The activity of antioxidants in midgut of larvae *Galleria mellonella* infected by *Bacillus thuringiensis***. IM Dubovskiy, EV Grizanova, EA Boiarisheva, VV Glupov, Lab of Insect Pathology, Institute of Animal Systematics and Ecology, Novosibirsk, Russia

3:45 142 **Assessing the non-target impacts of Foray 48B on soil biota**. M O'Callaghan[1], SU Sarathchandra[2], NL Bell[2], LTDavis[2], EM Gerard[1]. [1]AgResearch, Lincoln, New Zealand, [2]AgResearch, Hamilton, New Zealand

Wednesday, 2:00-4:00. BEB Lobby, 1st and 2nd floor

POSTERS – 2

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

VIRUSES

V-1 **Heterologous baculovirus pathogenicity in the absence of contemporary coevolution.** G Moreau[1], CJ Lucarotti[1], EG Kettela[1], KN Barber[2], SE Holmes[1], SB Holmes[2], C Weaver[1], B Morin[1]. [1]Natural Resources Canada, Canadian For Serv-Atlantic Forestry Centre, Fredericton, New Brunswick, Canada, [2]Natural Resources Canada, Canadian For Serv-Great Lakes Forestry 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 Moreau[1,2], E S Eveleigh[1,2], CJ Lucarotti[1,2], DT Quiring[2]. [1]Natural Resources Canada, Canadian For Serv - Atlantic Forestry Centre, Fredericton, New Brunswick, Canada, [2]Population Ecology Group, Faculty of Forestry and Environmental Management, Univ of New Brunswick, Fredericton, New Brunswick, Canada

V-3 **Efficacy of indigenous TnSNPV and AcMNPV isolates for control of *Trichoplusia ni*: Greenhouse cage trials.** M Erlandson[1,2], D Gillespie[3], M Strom[2], D Quiring[3], D Theilmann[3]. [1]Agriculture and Agri-Food Canada, Saskatoon Research Centre, and [2]Dept of Applied Microbiology and Food Science, Univ of Saskatchewan, Saskatoon, Canada, [3]Agriculture and Agri-Food Canada, Pacific Agri-Food Research Centre, Agassiz and Summerland, BC, Canada

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

V-5 **Aerosol infectivity of baculovirus to insect larvae: A new larval inoculation strategy for baculovirus.** T-Y Wu[1], T-R Jinn[2,3], S –S Kao[2], JTC Tzeng[3]. [1]Dept of Bioscience Technology, Chung Yuan Christian Univ, Chung Li, Taiwan, [2]Biopesticicde Dept, Taiwan Agriculture Chemicals and Toxic Substances Research Institute, Wufeng, Taiwan, [3]Graduate Institute of Biotechnology, National Chung Hsing Univ, Taichung, Taiwan

V-6 **Fall armyworm *Spodoptera frugiperda* base line of susceptibility to baculovirus SfNPV strain from Paraná, Brasil.** M Vázquez G[1], T López P[1], J Vázquez R[2]. [1]Lab de Entomología. División de Ciencias Agronómicas. Univ de Guadalajara, México, [2]División de Ciencias Biológicas, Univ de Guadalajara, México

V-7 **RAPD-PCR fragments marking resistance and susceptibility of *Lymantria dispar* to nuclear polyhedrosis virus**. AV Ivashov[1], AP Simchuk[1], VV Oberemok[1], VV Gouli[2]. [1]Dept of Ecology, VI Vernadsky Natl Univ, Ukraine [2]Entomology Research Laboratory, Dept of Plant and Soil Science, Burlington, VT USA

V-8 **Production of the *Lymantria dispar* nucleopolyhedrovirus in stirred tank bioreactors.** JM Slavicek, JM Gabler, USDA 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 *Erinnyis ello* granulovirus applied as a bioinsecticide in Brazil.** NR Costa[1], BC Ferreira[1], MEB Castro[1], R Pegoraro[2], ML Souza[1]. [1]Embrapa Genetic Resources and Biotechnology, Brasilia-DF, Brazil, [2]EPAGRI Santa Catarina S.A., Itajaí-SC, Brazil

V-11 **Coral red fluorescence protein as genetic modified baculovirus tracer.** T-R Jinn[1,2], S –S Kao[2], JTC Tzeng[1], T-Y Wu [3]. [1]Graduate Institute of Biotechnology, Natl Chung Hsing Univ, Taichung, Taiwan, [2]Biopesticicde Department, Taiwan Agriculture Chemicals and Toxic Substances Research Institute, Wufeng, Taiwan, [3]Dept of Bioscience Technology, Chung Yuan Christian Univ, Chung Li, Taiwan

V-12 **Short term starvation reduces intrastadial developmental resistance of gypsy moth (*Lymantria dispar*) to LdNPV**. J Harenza, M Grove, S Geib, 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 Liang[1,2], J Song[1,2], X Chen [1]. [1]State Key Lab of Virology, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan, [2]Graduate 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 Lung[1], GW Blissard[2]. [1]Dept of Biological Sciences, Univ of Lethbridge, Canada, [2]Boyce Thompson Institute, Cornell Univ, Ithaca, NY, USA

V-15 **Analysis of the CfMNPV IAP genes.** J de Jong[1], BM Arif[2], DA Theilmann[3], PJ Krell[1]. [1]Dept of Molecular and Cellular Biology, Univ of Guelph, ON, Canada, [2]Great Lakes Forestry Centre, Canadian Forest Service, Sault Ste. Marie, ON, Canada, [3]Agriculture and Agri-Food Canada, Summerland BC, Canada

V-16 STU **Screening of cellular factors which interact with Host Range Factor-1 (HRF-1) from *Lymantria dispar* nucleopolyhedrovirus**. H Ishikawa[1], M Ikeda[1], SM Thiem[2], M Kobayashi[1]. [1]Graduate School of Bioagricultural Sciences, Nagoya Univ, Chikusa, Nagoya, Japan, [2]Depts of Entomology and Microbiology and Molecular Genetics, Michigan State Univ, East Lansing, MI, USA

V-17 **Characterization of the *gp41* gene of *Spodoptera litura* multicapsid nucleopolyhedrovirus.** L Pan, Z Li, Y Gong, M Yu, K Yang, Y Pang, 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 polyhederosis virus.** Y Liu, 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 Wu[1], F Deng[1], X Sun[1], H Wang[1], L Yuan[1], JM Vlak[2], Z Hu[1]. [1]State Key Lab of Virology, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan, China, [2]Lab 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 nucleopolyhedrovirus genes following transfection of Tn5-B1 cells.** M van Munster[1], L Willis[2], M Elias[1], M Erlandson[3], D Theilmann[2], R Brousseau[1], L Masson[1].

[1]Biotechnology Research Institute, Natl Research Council of Canada, Montreal, Quebec Canada, [2]Pacific Agri-Food Research Centre, Summerland, BC, Canada, [3]Agriculture and Agri-Food Canada, Saskatoon Research Centre, and Dept of Applied Microbiology and Food Science, Univ of Saskatchewan, Saskatoon, Canada

V-22 **Organization of the *Choristoneura occidentalis* granulovirus genome.** SR Coppens, HAM Lauzon, PJ Krell[1], BM Arif, Great Lakes Forestry Centre, Sault Ste. Marie ON, [1]Dept of Microbiology, Univ of Guelph, ON, Canada

V-23 **The genome sequence of the *Mamestra brassicae* nucleopolyhedrovirus: An Old World virus compared with New World isolates.** SL Turner[1], JP Burden[1], C Ekeke[1], D Field[1], G Wilson[1], RS Hails[1], R Feldman[2], RD Possee[1]. [1]CEH Oxford, UK, [2]SymBio Corporation, Menlo Park, CA, USA

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

V-25 **Potential horizontal transfer of an insect trypsin-like serine protease to *Neopdiprion sertifer* NPV and *N. lecontei* NPV.** A Garcia-Maruniak[1], HAM Lauzon[2], BM Arif[2], CJ Lucarotti[3], JE Maruniak[1]. [1]Entomology and Nematology Dept, Univ of Florida, Gainesville, FL, USA, [2]Canadian Forest Service, Great Lakes Forestry Center, Sault Ste. Marie, Ontario, Canada, [3]Canadian Forest Service, Atlantic Forestry Centre, Fredericton, New Brunswick, Canada

V-26 **Multitemperature single-strand conformational polymorphism - a method for detection of minute changes in baculovirus genome.** B Szewczyk[1], P Barski[2], M Paluszek[1], L Hoyos-Carvajal[3], W Sihler[4], A Brzozowska[1], M Lobo de Souza[4]. [1]Dept of Molecular Virology, Intercollegiate Faculty of Biotechnology of the Univ of Gdansk and Medical Univ of Gdansk, Kladki, Poland, [2]A&A Biotechnology, Gdynia, Poland, [3]Instituto de Biología, Facultad de Ciencias Exactas y Naturales, Univ de Antioquia, Medellin, Colombia, [4]Embrapa Recursos Genéticos e Biotecnologia, Parque Estaçao Biológica, Brazil

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

V-28 STU **Searching for a homologue of the *Mythimna separata* entomopoxvirus gene encoding the protein lethal to the endoparasitoid *Cotesia kariyai*.** E Iizuka, S Okuno, M Nakai, T Ishii, J Takatsuka, K Nakanishi, 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: Lymantriidae) 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 aldolase expression in shrimps after white spot syndrome virus (WSSV) infection**. H-C Wang[1], H-C Wang[2], S-E Peng[2], C-F Lo[2], S-H Chiou[1]. [1]Institute of Biochemical Sciences, and [2]Institute of Zoology, National Taiwan Univ, Taipei, Taiwan

V-33 STU **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 STU **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 STU **Identification of basal promoter and enhancer regions in an untranslated region of WSSV *ie1*.** W-J Liu, C-F Lo, G-H Kou, Institute of Zoology, National Taiwan Univ, Taipei, Taiwan

MICROSPORIDIA AND PROTOZOA

MP-1 **The Eppendorf[R] - micromanipulator – a new technique for the quantitative separation of microspordian spores for infection experiments.** T Kolling[1], D Pilarska[2], A Linde[1]. [1]Fachhochschule Eberswalde, Dept of Forestry, Applied Ecology, Eberswalde, Germany, [2]Institute of Zoology, Bulgarian Academy of Sciences, Sofia, Bulgaria

MP-2 **A microsporidium infecting the black vine weevil, *Otiorhynchus sulcatus* (F.) (Coleoptera: Curculionidae).** DJ Bruck[1], L Solter[2]. [1]USDA-ARS Horticultural Crops Research Lab, Corvallis, OR, [2]Illinois Natural History Survey, Champaign, IL, USA

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 lepidoteran pests in Taiwan.** K Chin-Tai[1], C-C Tzeng[2], C-H Wang[1]. [1]Dept of Entomology, National Taiwan Univ, Taipei, Taiwan, [2]Division of Bio-Pesticide,Taiwan Agricultural Chemicals and Toxic Substances Research Institute, Council of Agriculture,Taichung, Taiwan

MP-5 **Complete sequence and secondary structure of ribosomal RNA gene of the *Nosema* sp. C 01.** J-Y Choi[1], J-G Kim[1], Y-C Choi[1], W-T Kim[1], H-S Sim[1], J Wuyts[2], Y-H Je[3]. [1]Dept of Sericulture and Entomology, National Institute of Agricultural Science and Technology, Republic of Korea, [2]Dept of Plant Biology, Flander Interuniv Institute for Biotechnology (VIB), Ghent Univ, Ghent, Belgium, [3]Dept of Agricultural Biotechnology, Seoul National Univ, Republic of Korea

MICROBIAL CONTROL

MC-1 **Intraguild interactions between *Verticillium lecanii* (Zimmermann) Viegas and *Aphidoletes aphidomyza* (Diptera: Cecidomyiidae) as biological control agents of *Myzus persicae* (Homoptera: Aphididae)**. P Jaramillo[1], B Roitberg[1], M Goettel[2], D Gillespie[2]. [1]Dept of Biological Sciences, Simon Fraser Univ, [2]Agriculture and Agri-Food Canada, Canada

MC-2 **Effects of *Verticillium lecanii* (*Lecanicillium* spp) against two-spotted spider mite, *Tetranychus urticae* and its natural enemy *Phytoseiulus persimilis***. M Koike[1], T Kodama[1], A Kikuchi[1,2], M Okabe[1], K Kuramoti[1], Y Saito[2]. [1]Dept of Agro-environmental Science, Obihiro Univ of Agriculture and Veterinary Medicine, Hokkaido, Japan, [2]Dept of Ecology and Systematics, Hokkaido Univ, Sapporo Japan

MC-3 **Factors that influence the desiccation tolerance and storage stability of blastospores of the entomopathogenic fungus *Paecilomyces fumosoroseus***. MA Jackson[1],S Cliquet[2], S Erhan[3], WJ Connick, Jr[4]. [1]USDA-ARS, Natl Center for Agricultural Utilization Research, Peoria, IL, USA, [2]Lab Univ de Microbiologie Appliquée de Quimper, France, [3]Georgia-Pacific Corporation, IL, USA, [4]USDA-ARS, New Orleans, LA, USA

MC-4 **Considerations in using *Metarhizium anisopliae* as a biopesticide for wireworms.** JT Kabaluk[1], MS Goettel[2], RS Vernon[1]. [1]Agriculture and Agri-Food Canada - Pacific Agri-Food Research Centre, Agassiz, BC, [2]Lethbridge Research Centre, Alberta, Canada

MC-5 **Virulence of fungal biocontrol agent *Beauveria bassiana* to the eggs and adults of carmine spider mite *Tetranychus cinnabarinus*.** W-B Shi[1], M-G Feng [1, 2], [1]Institute of Applied Entomology, Coll of Agriculture and Biotechnology, Zhejiang Univ, PR China, [2]Institute of Microbiology, College of Life Sciences, Zhejiang Univ, PR China

MC-6 **Development of *Beauveria bassiana*-based mycoinsecticide for tea leafhopper control in China: Current status and prospects.** M-G Feng [1, 2], S-H Ying[1]. [1]Institute of Microbiology, College of Life Sciences, Zhejiang Univ, PR China, [2]Institute of Applied Entomology, Coll of Agriculture and Biotechnology, Zhejiang Univ, PR China

MC-7 **Modeling analysis of the interaction of *Beauveria bassiana* and imidacloprid on two aphid pests.** S-D Ye [1], M-G Feng[1, 2]. [1]Institute of Microbiology, College of Life Sciences, Zhejiang Univ, PR China, [2]Institute of Applied Entomology, College of Agriculture and Biotechnology, Zhejiang Univ, PR China

MC-8 **Quantified interaction of fungal biocontrol agent *Beauveria bassiana* and a thiosultap-diaminium insecticide on *Plutella xylostella* larvae.** L Tian[1], M-G Feng[1,2]. [1]Institute of Microbiology, and [2]Institute of Applied Entomology, Zhejiang Univ, PR China

MC-9 **Susceptibility of larval stages of the aphid parasitoids *Aphidius colemani* and *A. matricariae* to the entomopathogenic fungus *Beauveria bassiana*.** M Filotas[1], J Sanderson[1], S Wraight[2]. [1]Dept of Entomology, Cornell Univ, and [2]USDA-ARS, US Plant, Soil, & Nutrition Laboratory, Ithaca, NY, USA

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*.** M Filotas[1], J Sanderson[1], S Wraight[2]. [1]Dept of Entomology, Cornell Univ, and [2]USDA-ARS, US Plant, Soil, & Nutrition Laboratory, Ithaca, NY, USA

MC-11 **STU** **Toxicity analysis of truncated insecticidal crystal proteins Cry1Ba3 from *Bacillus thuringiensis*.** G Wang[1], J Zhang[1], J Wu[1], F Song[1], D Huang[2]. [1]State Key Lab for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, and [2]Institute of Biotechnology Research, Chinese Academy of Agricultural Sciences, Beijing, PR China

MC-12 **Is phenoloxidase involved in induced resistance to *Bacillus thuringiensis kurstaki* in *Trichoplusia ni*?** AF Janmaat[1,3], J Manson[2,3], J Ericsson[3], V Caron[3], JH Myers[3]. [1]Dept of Biological Sciences, Simon Fraser Univ, Burnaby, BC, Canada, [2]Dept of Zoology, Univ of Toronto, Ontario, Canada, [3]Dept of Zoology, Univ of British Columbia, Vancouver, BC, Canada

MC-13 **STU** **Construction of a *Bacillus thuringiensis* BAC library and partial cloning of zwittermicin A biosynthesis cluster.** T Shao[1,3] F Song[1] J Zhang[1] D Liu[3] D Huang[1,2]. [1]State Key Lab of Biology for Plant Diseases and Insect Pests, Institute of Plant Protection, and [2]Institute of Biotechnology Research, Chinese Academy of Agricultural Sciences, Beijing, [3]Agricultural Univ of HeBei, BaoDing, PR China

MC-14 **Implementation of the largest worldwide laboratory production of a baculovirus: The case of the nucleopolyhedrovirus of *Anticarsia gemmatalis* (Lep.: Noctuidae) in Brazil.** F Moscardi[1], B Santos[2]. [1]Embrapa Soja, Londrina, PR, Brazil, [2]Univ Federal do Parana, Curitiba, PR, Brazil

MC-15 **Laboratory and orchard studies on the transmission of *Cydia pomonella* granulovirus by contaminated *C. pomonella* adults.** D Winstanley[1], JV Cross[2], N Naish[1], G Keane[1], S Hilton[1], D Gajek[2], R van Wezel[2], [1]Warwick HRI, Wellesbourne, Warwick UK, [2]East Malling Research, West Malling, Kent, UK

NEMATODES

N-1 **Evaluation of a native *Heterorhabditis* species from the Coastal Region of Central Perú against white grubs.** J Alcázar[1], C Farfán[2], J Salazar[1], C Castillo[1], HK Kaya[3]. [1]International Potato Center, Lima, Perú, [2]Institute Valle Grande, Cañete, Perú, [3]Dept of Nematology, Univ of California, Davis, CA, USA

N-2 **Targeting the Andean weevils with a native entomopathogenic nematode species.** S Parsa[1], J Alcazar[2], L Lizarraga[3], HK Kaya[1]. [1]Dept of Nematology, Univ of California, Davis, CA, USA, [2]International Potato Center, Lima, Peru, [3]CRIBA, Univ of Cusco, Cusco, Peru

N-3 **Virulence of various commercial isolates of *Heterorhabditis bacteriophora* against the European chafer (*Rhizotrogus majalis*).** L Simard[1], G Bélair[1], J Dionne[2]. [1]Horticulture Research and Development Centre, Agriculture and Agri-Food Canada, Quebec, Canada, [2]Royal Canadian Golf Association, Oakville, Ontario, Canada

N-5 **STU** **Infectivity of entomopathogenic nematodes and immune responses of their insect hosts.** X Li[1], RS Cowles[2], E Cowles[3], R Gaugler[4], DL Cox-Foster[1]. [1]Dept of Entomology, The Pennsylvania State Univ, PA, [2]The Connecticut Agricultural Experiment Station, Windsor, CT, [3]Dept of Biology, Eastern Connecticut State Univ, Willimantic, Connecticut, [4]Dept of Entomology, Rutgers Univ, New Brunswick, NJ, USA

OTHER

O-1 **The *Sleeping Beauty* transformation system: A new approach for the study of tick cell microbe interactions.** TJ Kurtti, RF Felsheim, GD Baldridge, NY Burkhardt, MJ Herron, UG Munderloh, Dept of Entomology, Univ of Minnesota, St. Paul, MN, USA

4:00–4:30	BREAK	BEB Lobby

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-Lorena, 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 *Po*GV and *Bacillus thuringiensis* (Berliner) against the potato tuber moth, *Phthorimaea operculella* (Zeller).** M Sporleder[1], D Mamani[2], J Huber[3], J Kroschel[1]. [1]International Potato Center, Lima, Peru, [2]Univ Nacional Mayor de San Marcos, Lima, Peru, [3]Institute 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 Lacey[1], S Arthurs[1], HL Headrick[1], R Fritts, Jr[2]. [1]USDA-ARS, Yakima Agricultural Research Lab, Wapato, WA, [2]Certis USA, Clovis, CA, USA

5:00 149 **Semiochemical driven auto-dissemination of viruses for the control of orchard pests.** D Winstanley[1], JV Cross[2], N Naish[1], G Keane[1], S Hilton[1], D Gajek[2], R van Wezel[2]. [1]Warwick HRI, Univ of Warwick, Wellesbourne, UK, [2]East Malling Research, West Malling, Kent, UK

5:15 150 **Biotic and abiotic factors affecting the field persistence and residual efficacy of *Cryptophlebia 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 Kleinschafer, 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 Jung, Federal Biological Research Centre for Agriculture and Forestry, Institute for Biological Control, Darmstadt, Germany

6:15 STU 154 **Construction of the rfp gene marker system to monitor insecticidal and anti-fungal engineered bacterium of *Pseudomonas fluorescens* Biop8**. Y Jia[1,2], J Zhang[1], G Li[3]. [1]State Key Laboratory of Biology for Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, [2]Agricultural Univ of HeBei, BaoDing, and [3]Agricultural College of LaiYang, PR China

Contributed Papers Wednesday, 4:30-6:30. BEB 101

VIRUSES 3

Moderator: Rollie Clem.

4:30 155 **Specific binding of AcMNPV ODV to midgut cellular targets is mediated by *pif* 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 STU 156 **Impact of the peritrophic matrix on baculoviral pathogenesis in a tritrophic system.** RC Plymale, K Hoover**,** Dept of Entomology, Pennsylvania State Univ, University Park, PA, USA

5:00 STU 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 STU 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 NeabNPV-infection in Balsam Fir Sawfly, *Neodiprion abietis* larvae.** B Whittome[1], B Morin[2], C Lucarotti[2], D Quiring[3], D Levin[1]. [1]Univ of Victoria, Victoria, BC, Canada, [2]Natural Resources Canada, Canadian Forestry Service, and [3]Faculty of Forestry and Environmental Management, Univ of New Brunswick, Fredericton, NB, Canada

5:45 160 **Characterization of *Helicoverpa armigera* nucleopolyhedrovirus ORF2**. Y Nie[1,2], Q Wang[1,3], C Liang[1,3], Z Yu[2], X Chen[1]. [1]State Key Lab of Virology, Wuhan Institute of Virology, Chinese Academy of Virology, Wuhan, [2]Institution of Entomology, Central China Normal Univ, Wuhan, [3]Graduate 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 Goley[1], T Ohkawa[2], M Welch[1], L Volkman[1,2]. [1]Dept of Molecular and Cell Biology, and [2]Dept 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 Dai[1,2], BM Arif[3], PJ Krell[2], DA Theilmann[1]. [1]Pacific Agri-Food Research Centre, Agriculture and Agri-Food Canada, Summerland, BC, Canada, [2]Dept of Molecular and Cellular Biology, Univ of Guelph, Ontario, Canada, [3]Great 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

SIP Division Business Meetings: Wednesday evening

Bacteria (6:45-7:45p) Rm 106 HC

Nematodes (6:45-7:45p) Canary Rm, HC

Microbial Control (7:45-8:30p) Rm 107 HC

Microbial Control Div. Workshop, Wednesday, 8:30-9:30. Rm 107 HC

Bioassays with insect pathogens: Concepts and performance

Organizers: Jørgen Eilenberg, Michael McGuire, Jeff Lord.

8:30 163 **Methods for analysis of data from bioassays with insect pathogens**. J Throne, USDA-ARS, Manhatten, Kansas, USA

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

THURSDAY - 11 August

Symposium (Microsporidia Division) Thursday, 8:00–10:00. BEB 111

Why study Microsporidia? Interesting research areas besides taxonomy and biological control

Organizers/Moderators: Gernot Hoch and Leellen Solter.

8:00 164 **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 *Brachiola* and human skeletal muscle infection caused by the mosquito microsporidium, *B. algerae***. A Cali, Dept of Biological Sciences, Rutgers Univ, Newark, NJ, USA

Contributed Papers Thursday, 8:00-10:00. BEB 101

VIRUSES 4

Moderator: Lorena Passarelli.

8:00 168 **Establishment of a natural phylogeny of Baculoviruses.** R Hauschild[1], M Lange[1], O Bininda-Emonds[2], JA Jehle[1]. [1]Labor für Biotechnologischen Pflanzenschutz, Dienstleistungszentrum Ländlicher Raum Rheinpfalz, Breitenweg, Germany, [2]Lehrstuhl für Tierzucht, Technische Univ München, Freising-Weihenstephan, Germany

8:15 STU 169 **Whole genome sequence analysis of a Polish isolate of *Agrotis segetum* nucleopolyhedrovirus.** AK Jakubowska[1,2], RMK Lankhorst[3], J Ziemnicka[1], JM Vlak[2], MM van Oers[2]. [1]Dept of Biological Control and Quarantine, Institute of Plant Protection, Poznań, Poland, [2]Lab of Virology, Wageningen Univ, and [3]Greenomics, Plant Research International, Wageningen, The Netherlands

8:30 170 **Genome sequence and organization of the *Neodiprion abietis* nucleopolyhedrovirus.** S Duffy[1], B Morin[2], C Lucarotti[2], D Levin[1]. [1]Univ of Victoria, Victoria, BC, Canada, [2]Natural Resources Canada, Canadian Forestry Service, Fredricton NB, Canada.

8:45 171 **Morphological, molecular, and genomic characterization of two mosquito Cypoviruses.** TB Green[1], A Sharpio[1], S White[1], S Rao[2], P Mertens[2], G Carner[3], JJ Becnel[1], [1]USDA-ARS, Center for Medical, Agricultural and Veterinary Entomology, Gainesville, FL, [2]Pirbright Lab, Institute for Animal Health, Woking, Surrey, UK, [3]Clemson Univ, Clemson, SC, USA

9:00 172 **Integration of an ichnovirus genome segment in the genomic DNA of lepidopteran cells.** D Doucet[1], A Levasseur[1], C Béliveau[1], D Stoltz[2], M Cusson[1]. [1]Laurentian Forestry Centre, Sainte-Foy, QC, Canada, [2]Dept 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 Lapointe[1], BA Webb[2], K Tanaka[2], W Barney[2], D Stoltz[3], M Cusson[1]. [1]Laurentian Forestry Centre, Sainte-Foy, QC, Canada, [2]Dept of Entomology, Univ of Kentucky, Lexington, USA, [3]Dept of Microbiology, Dalhousie Univ, Halifax, Canada

9:30 174 **Display of a foreign protein using recombinant baculovirus occlusion bodies: A novel vaccination tool.** R Wilson[1], Y Je[1], L Bugeon[1], U Straschil[1], DR O'Reilly[1], JA Olszewski[1,2]. [1]Dept of Biological Sciences, Imperial College London, UK, [2]Biology Dept, Shippensburg Univ, PA, USA

9:45 175 **flashBAC: A baculovirus expression system for automated, high throughput production of proteins.** L King[1], K Richards[1], R Hitchman[1], H Irving[1], S Mann[1], E Siaterli[1], R Possee[2]. [1]School of Biological and Molecular Sciences, Oxford Brookes Univ, UK, [2]NERC 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 Liu[1], LS Bauer[1,2]. [1]Dept of Entomology, Michgian State Univ, and [2]USDA Forest Service, North Central Research Station, E. Lansing, MI, USA

8:15 STU 177 **A proactive approach to the use of fungal biopesticides to manage sucking insects in pulse crops in Australia.** K Knight[1,2], C Hauxwell[1], D Holdom[1], G Simpson[3]. [1]DPI&F Biopesticides Unit, Indooroopilly, [2]School of Integrative Biology, Univ of Queensland, St Lucia, Australia, [3]DPI&F, Toowoomba, Queensland, Australia

8:30 178 **Evaluation of some hyphomycetous fungi for the control of glassy-winged sharpshooter, *Homalodisca coagulata* (Homoptera: Cicadellidae)**. SK Dara[1], MR McGuire[2], HK Kaya[3]. [1]Shafter Research and Extension Center CA, [2]USDA-ARS, Shafter, CA, [3]Dept of Nematology, Univ of California, Davis, CA, USA

8:45 179 **Field testing of selected *Beauveria bassiana* isolates against *Lygus hesperus* in California**. MR McGuire[1], JE Leland[2]. [1]USDA-ARS, Shafter, CA, USA, [2]USDA-ARS, Stoneville, MS, USA

9:00 180 **Selection and field evaluation of *Beauveria bassiana* isolates for control of tarnished plant bug, *Lygus lineolaris*.** JE Leland[1], MR McGuire[2], J Gore[1]. [1]USDA-ARS, SIMRU, Stoneville, MS, [2]USDA-ARS, SREC, Shafter, CA, USA

9:15 181 **Fungal BCAs: Potential control agents to control subterranean pests**. H Strasser[1], B Pernfuss[1], RK Morelli[2]. [1]Institute of Microbiology, Leopold Franzens Univ Innsbruck, Austria, [2]Agrifutur Srl, Brescia, Italy

9:30 STU 182 **To germinate or not? Strategies of *Beauveria bassiana* for survival in soil.** CV Mander[1], TA Jackson[2], B Chapman[1]. [1]Bio-Protection and Ecology Division, Lincoln Univ, and [2]AgResearch, Lincoln, Canterbury, New Zealand

9:45 STU 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

Contributed Papers Thursday, 8:00-10:00. BEB 117

BACTERIA 3

Moderator: Neil Crickmore.

8:00 184 **Variability of fitness costs associated with Cry1A resistance in *Helicoverpa armigera* on cotton and alternative refuge crops**. L Bird, R Akhurst. CSIRO Entomology, Canberra, Australia

8:15 STU 185 **Genetic response of the Spruce budworm *Choristoneura fumiferana*, to sublethal *Bacillus thuringiensis* Cry1Ab toxin exposure.** L Meunier[1], G Préfontaine[1],Q Feng[2], R Brousseau[1], L Masson[1]. [1]Natl Research Council of Canada, Biotechnology Research Institute, Montreal, Quebec, Canada, [2]Great Lakes Forestry Centre, Canadian Forest Service, Sault Ste. Marie, Ontario, Canada

8:30 186 **Effects of entomopathogenic nematodes on the fitness cost of resistance to *Bacillus thuringiensis* in the pink bollworm**. AJ Gassmann[1], SP Stock[2], Y Carrière[1], BE Tabashnik[1]. [1]Dept of Entomology, Univ of Arizona, Tucson, AZ, [2]Division of Plant Pathology and Microbiology, Dept of Plant Sciences, Univ of Arizona, Tucson, AZ, USA

8:45 STU 187 **Identification and characterization of *Bacillus thuringiensis* strains by molecular methods.** GV Kalmykova,[1], LI Burtseva[1], AM Lysenko[2]. [1]Lab of Insect Pathology, Institute of Animal Systematics and Ecology, SB RAS, Novosibirsk, Russia, [2]Institute of Microbiology, RAS, Moscow, Russia

9:00 STU 188 **Isolation, molecular characterization and insecticide potential of *Bacillus thuringiensis* strains isolated from Madurae dt. (Tamilnadu).** A Mahalakshmi, R Shenbagarathai, K Sujatha, Dept of Zoology and Research Centre, Madurai, Tamilnadu, India

9:15 189 **The expansion of *Bacillus thuringiensis* subsp. *toguchini* in the environment.** VP Khodirev, Institute of Animal Systematics and Ecology, SB RAS, Novosibirsk, Russia

9:30 STU 190 **Targeted delivery of genetically conjugated Cyt1Aa from *Bacillus thuringiensis* subsp. *israelensis* into myeloma model cells.** S Cohen[1,2], E Ben-Dov[1], M Nisnevitch[2], R Cahan[2], M Firer[2], A Zaritsky[1]. [1]Dept of Life Sciences, Ben-Gurion Univ of the Negev, Be'er-Sheva, Israel, [2]Dept of Chemical Engineering and Biotechnology, College of Judea and Samaria, Ariel, Israel

9:45 STU 191 **Differential expression of cry toxin in a *Bacillus thuringiensis* strain with dual insecticidal activity**. J Torres[1], NA Valdez-Cruz[1], JD Tinoco[2], S Orduz[1,3]. [1]Unidad de Biotecnología y Control Biológico, Corporación para Investigaciones Biológicas, Carrera, Colombia, [2]Coltabaco, Compañía Colombiana de Tabaco S. A., Colombia, [3]Univ de Pamplona, Pamplona, Santander, Colombia

10:00–10:30 BREAK BEB Lobby

Thursday, 10:30-12:30. Cuddy Center

SOCIETY for INVERTEBRATE PATHOLOGY
Annual Business Meeting

Presiding: Just Vlak.

12:30–1:30 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 Biotecnologia, 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: Parwinder 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

Contributed Papers Thursday, 1:30-3:30. BEB 117

VIRUSES 5

Moderator: David Theilmann.

1:30 204 **On the analogy of the baculovirus and whispovirus DNA binding proteins.** M Westenberg, J Witteveldt, E Tuladhar, MF Boyong, JM Vlak**,** Lab of Virology, Wageningen Univ, Wageningen, The Netherlands

1:45 205 **Replication in *Trichoplusia ni* larvae of AcMNPV mutants that express only IE0 or IE1.** MA Erlandson[1],TM Stewart[3], LG Willis[2], DA Theilmann[2,3]. [1]Saskatoon Research Centre, Agriculture and Agri-Food Canada, Canada, [2]Pacific Agri-Food Research Centre, Agriculture and Agri-Food Canada, Summerland, BC, Canada, [3]Faculty of Agricultural Sciences, Univ of British Columbia, Vancouver, BC, Canada

2:00 206 **A common net work for the activation of early promoters through baculovirus polyhedrin upstream sequence**. CPY Wu, Y-C Chao, Institute of Molecular Biology, Academia Sinica, Nankang, Taipei, Taiwan

2:15 207 **Functional characterization of BmNPV and Se*M*NPV late gene transcription and genome replication factors in the insect cell line SF-21**. MF Berretta, M Deshpande, AL Passarelli. Division of Biology, Kansas State Univ, Manhattan, KS, USA

2:30 208 **Oral infection of *Spodoptera exigua* larvae with an AcMNPV mutant lacking the apoptosis suppressor p35**. RJ Clem[1], L Heaton[1], M Burton[2]. [1]Division of Biology and [2]Dept of Anatomy and Physiology, Kansas State Univ, Manhattan, KS, USA

2:45 209 **Identification and functional analysis of *Leucania separata* multiple nuclear polyhedrosis virus *iap3* and *p49* genes in sf9 cells.** E Du, W Jin, W Zhou, F Yan, Y Qi. Institute of Virology, Wuhan Univ, PR China

3:00 210 **Characterization of a novel entomopoxvirus homolog of baculovirus P35.** JC Means, RJ Clem, Molecular, Cellular, and Developmental Biology Program, Division of Biology, Kansas State Univ, Manhattan, KS, USA

3:15 211 **ORF390 of white spot syndrome virus genome is identified as a novel anti-apoptosis gene.** Y Qi, H Xu, Z Wang, Q Zhou, L Hu, L Yao, State Key Lab of Virology, Wuhan Univ, Wuhan, Hubei Province, PR China

3:30–4:00 BREAK BEB Lobby

Symposium (Div. of Viruses) Thursday, 4:00-6:00. BEB 101

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 Bergoin, Lab de Pathologie Comparee, Univ Montpellier II, Montpellier, France

5:00 214 **BacMam viruses: Versatile tools for mammalian cell-based assay development**. P Condreay, Gene Expression and Proteins Biochemistry, GlaxoSmithKline Discovery Research, North Carolina, USA

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

Contributed Papers Thursday, 4:00-4:45. BEB 117

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[1], C Berry[2], Y Yang[2], WE Walton[1], BA Federici[1]. [1]Dept of Entomology, Univ of California, Riverside, CA, USA, [2]Cardiff School of Biosciences, Cardiff Univ, Wales, UK

4:30 218 **Pore-forming determinants of *Bacillus thuringiensis* Cry4 mosquito-larvicidal proteins.** C Angsuthanasombat, Lab of Molecular Biophysics and Structural Biochemistry, Institute of Molecular Biology and Genetics, Mahidol Univ, Thailand

Contributed Papers Thursday, 4:00-5:30. BEB 110

MICROBIAL CONTROL 3

Moderator: Helen Roy and Judith Pell.

4:00 219 **Host plant determines efficacy of *Beauveria bassiana* against western flower thrips.** TA Ugine[1], SP Wraight[2], JP Sanderson[1]. [1]Dept of Entomology, Cornell Univ, USA, [2]USDA-ARS, US Plant Soil and Nutrition Laboratory, USA

4:15 220 ***Beauveria bassiana* and *Fusarium oxysporum* as endophytes in banana tissue culture plants**. T Dubois[1], CS Gold[1], P Paparu[1], J Akello[1], E Adipala[2], D Coyne[1]. [1]Intl Institute of Tropical Agriculture, Southern and Eastern Africa Regional Centre, Namulonge, and [2]Dept of Crop Science, Makerere Univ, Kampala, Uganda

4:30 221 **Microbial control of the banana weevil, *Cosmopolites sordidus*, with *Beauveria bassiana***. CS Gold[1], C Nankinga[1,2], W Tinzaara[1], T Dubois[1], J Akello[1], W Tushemereirwe[2]. [1]Intl Institute of Tropical Agriculture, Southern and Eastern Africa Regional Centre, Namulonge, and [2]Uganda National Banana research Programme, Kampala, Uganda

4:45 222 **Effects of day versus evening application times on efficacy of *Beauveria bassiana* foliar sprays against Colorado potato beetle**. S Wraight, ME Ramos, USDA-ARS-PPRU, US Plant, Soil, and Nutrition Lab, Ithaca, New York, USA

5:00 223 **Use of genetic diversity in *Beauveria bassiana* for improving the biological control of the coffee berry borer.** LP Cruz[1], AL Gaitan[2], CE Gongora[1]. [1]Dept of Entomology, and [2]Dept of Plant Pathology, Natl Centre of Coffee Research, Chinchina, Caldas, Colombia

5:15 224 **The application of *Metarhizium anisopliae* and *Beauveria bassiana* for the control of the longicorn beetle borer *Agrianome spinicolis* (Cerambycidae) in pecan trees.** IR Newton[1], A Ward[2]. [1]Stahmann Farms, Trawalla, Pallamallawa, NSW, Australia, [2]Becker Underwood, Somersby, NSW, Australia

5:30 225 **Microbial control of the spotted stemborer *Chilo partellus* with *Beauveria bassiana* and *Metarhizium anisopliae* from Ethiopia and South Africa**. T Tefera, Alemaya Univ, Dept of Plant Sciences, Ethiopia

7:00p–1:00a	BANQUET & AWARDS CEREMONY	Captain Cook Hotel
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	

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in Wuhan!

ABSTRACTS

2005

IMPORTANT NOTES:

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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

MONDAY - 8 August

PLENARY SYMPOSIUM. Monday, 10:30–12:30

Invertebrate pathogens: Evolution and impact

Symposium. Monday, 10:30. 1

One step ahead of emerging crustacean viruses

Chu-Fang Lo 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, Whispovirus, 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 anti-disease 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. 2

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, 11:30. 3

All models are wrong, but some models are useful: Using mechanistic models to understand insect pathogens

Greg Dwyer

Dept. of Ecology and Evolution, U. Chicago, 1101 E 57th St, Chicago IL 60637-1573, USA

Mathematical models have long been used to generate qualitative insights about disease spread. Increasingly, however, models are also used as statistical tools for understanding data, and thus as testable, quantitative hypotheses. Work in my lab uses models to understand pathogens of the gypsy moth, *Lymantria dispar*. By adding stochasticity to an existing disease model, we have developed a method of using infection rate data to make inferences about mechanisms of disease spread. This work has suggested that heterogeneity in susceptibility among gypsy moth larvae plays an important role in NPV transmission. We are extending this approach to disentangle the effects of the NPV and the fungal pathogen *Entomophaga maimaga* on gypsy moth population dynamics. In addition, we are developing methods of using models to make inferences about NPV sequence data. This latter work has suggested first that geographic separation plays only a small role in the evolution of this virus. Also, our data have shown that, at the *25K fp* locus, which affects polyhedron size and number, there is a high-frequency polymorphism. By allowing for pathogen variability in our models, we are attempting to understand the consequences of this polymorphism for the spread of the NPV.

Symposium. Monday, 12:00. 4

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 (*Yersinia*, *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.

SYMPOSIUM (Cross-Divisional). Monday, 2:00-4:00

Diseases of marine invertebrates

Symposium. Monday, 2:00. 5

***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 (*Carcinus maenas*) and harbor (*Portunus 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 *Tiostrea chilensis*, 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 infected *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. Reece[1], Jessica A. Moss[1], Nancy A. Stokes[1], Ryan B. Carnegie[1], Christopher Dungan[2] and Eugene M. Burreson[1]

[1]Virginia Institute of Marine Science, The College of William and Mary, Gloucester Point, VA 23062, and [3]Maryland 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 rickettsiae, "*Candidatus Xenohaliotis californiensis*" which infects gastroepithelial 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 and tissue changes were noted between species. Black, *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-medicated 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.

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

FUNGI

Contributed paper. Monday, 2:00. 9

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.

Contributed paper. Monday, 2:15. 10

Reduced susceptibility of over-wintering ladybirds to *Beauveria bassiana*

Helen Roy[1], Emma Ormond[1] and Michael Majerus[2]

[1]Department of Life Sciences, Anglia Polytechnic University, Cambridge, CB1 1PT, [2]Department of Genetics, Downing Street, Cambridge, CB2 3EH, UK

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. *Coccinella 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.

Contributed paper. Monday, 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)

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 entomophtoralean 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 hyphomycetous fungi, *Beauveria bassiana* and *Lecanicillium 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 mm^2. 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 *D. noxia, R. padi, Galleria mellonella* or *Tenebrio molitor*. Results are discussed.

Contributed paper. Monday, 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

Louela A. Castrillo[1], Michael H. Griggs[2], Eleanor Groden[3], Seanna L. Annis[3], Prashant K. Mishra[3] and John D. Vandenberg[2]

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

Our study on the impact of inundative releases of *Beauveria bassiana* GHA mycoinsecticides on indigenous conspecific populations revealed persistence of the GHA strain in the field four years at most since the last application. Molecular analysis of recovered GHA "clones" using AFLP markers also revealed detectable genetic changes distinguishing the field collected isolates from the sprayed technical products. Because genetic stability is important in assessing environmental risks of an introduced fungal entomopathogen, any genetic change and, consequently, correlated phenotypic changes, especially virulence and host range, need to be examined for non-target effects. In this study we examined virulence changes by conducting bioassays against the Colorado potato beetle of three GHA field-recovered "clones" versus a GHA technical product. Results showed that strains A18 and FA1 were of comparable virulence to the technical product, while strain G30 had significantly lower virulence. Among the GHA-like strains, A18 and FA1 were collected a year after the last GHA application and were genetically very similar to the technical product. In contrast, G30, collected four years after the last spray, has mixed ancestry, suggesting that the lower virulence in this strain may be due to recombination between GHA and an indigenous isolate.

Contributed paper. Monday, 3:00. 13

Identification and characterization of genes responsible for pathogenicity of *Beauveria bassiana* towards the coffee berry borer

Javier G. Mantilla[1], Alvaro L. Gaitan[2] and Carmenza E. Gongora[1]

[1]Department of Entomology, and [2]Department of Plant Pathology, CENICAFE (National Centre of Coffee Research), Chinchina, Caldas, Colombia

To identify pathogenicity genes from the fungus *Beauveria bassiana* towards the coffee berry borer (*Hypothenemus hampei*), differential libraries from the strain Bb 9205 were constructed by subtractive hybridization. RNA populations were obtained after 24 hours of spore germination and fungal growth under two conditions: SDB medium plus 1% yeast extract (driver) and Minimal medium plus 10% w/v coffee berry borer (tester). A total of 174 clones were sequenced with a redundancy of 46%. A Unigene set of 94 unique sequences, with an average size of 600bp, was compared to the GenBank using BLASTn and BLASTx. For 36 sequences (38%) no homologies were found in the database, while 58 (62%) showed significant homology, mostly to proteins or hypothetical proteins. Only a phosphoenolpyruvate carboxykinase (PEPCK1) gene was previously identified from *B. bassiana*. The most abundant sequences corresponded to hypothetical proteins that encode for a peroxysomal copper amine oxidase and a cyanamide hydratase (urea hydro-lyase). Among the genes found, there is a homolog of the protease PR1J subtilisin-like serine from *Metarhizium anisopliae*, reportedly associated to pathogenicity and invasion processes towards insects. This research, presents the *B. bassiana* genome as an interesting source for further biotechnological applications.

Contributed paper. Monday, 3:15. 14

Germination polarity of conidia and its correlation with pathogenicity of *Beauveria bassiana* isolates

Reza Talaei-hassanloui[1,2], Aziz Kharazi-pakdel[1], Mark S. Goettel[2], Shannan Little[2], and Javad Mozaffari[3]

[1]Dept of Plant Protection, College of Agriculture, University of Tehran, Karaj, 31584-11187, Iran, [2]Lethbridge Research Centre, 5403-1 Avenue South, P.O.Box 3000, Lethbridge, Alberta T1J 4B1, Canada, and [3]Dept of Genetics, Seed and Plant Improvement Institute, Karaj, Iran

Three different germination types of conidia were revealed through microscopic observations *in vitro* and *in vivo* for 10 *Beauveria bassiana* isolates. These were classified as either uni-directional, bi-directional or multi-directional germination. *In vitro* conidial germination assessment on SDAY demonstrated that there are significant differences in germination polarity of conidia among isolates. Canonical correlation analysis indicated that there is a positive correlation (Pcc =0.71, Bonferroni), *Plutella xylostella* and

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 Nielsen[1], Melody Keena[2], and Ann E. Hajek[1]

[1]Department of Entomology, Cornell University, Ithaca, NY 14853, USA, [2]USDA 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. maimaga* 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 Hjeljord[1] and Ingeborg Klingen[2]

[1]Norwegian University of Life Sciences, P.O. Box 5003, N-1432 Aas, Norway, [2]The 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 (Ma), 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°C) against three pest insects: *Otiorhynchus sulcatus* (Coleoptera), *Operopthera 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 (±23.2)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.

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

BACTERIA

Contributed paper. Monday, 2:00. 17

Characterization of the cadherin protein from *Lymantria dispar* as Cry1A toxin receptor

Juan L. Jurat-Fuentes[1], Algimantas P. Valaitis[2] and Michael J. Adang[1,3].

Departments of [1]Entomology and [3]Biochemistry & Molecular Biology, University of Georgia, Athens, GA 30602, USA, [2]USDA Forest Service, Delaware, Ohio 43015, USA

Cadherin-like proteins from *Bombyx mori*, *Manduca sexta*, *Pectinophora gossypiella*, and *Heliothis virescens* have been, directly or indirectly, demonstrated to function as receptors for Cry1A toxins. The objective of this work was to test the putative role of the cadherin protein from *Lymantria dispar* (LdCad) as receptor for Cry1A toxins. Two approaches were followed to characterize Cry1A-LdCad interactions. First, truncated LdCad proteins containing regions predicted to be involved in toxin binding were expressed in *Escherichia coli*. Cry1A toxin binding to these LdCad truncated peptides was tested using ligand blotting, dot blotting and affinity chromatography. In a second approach, full length LdCad was expressed in S2 and High Five insect cell cultures. Expressed LdCad was detected on the surface of the transfected cells by immunocytochemistry. Binding of Cry1A toxins to cells expressing LdCad was tested using dot blotting and fluorescence microscopy. Fluorescence assisted cell sorting (FACS) was used to test cytotoxicity of Cry1A toxins against cells expressing LdCad. In these assays, all Cry1A toxins (but not Cry1Fa) killed cells expressing LdCad, with Cry1Ac being the most active toxin. These results are evidence for a functional Cry1A receptor role for LdCad

STU Contributed paper. Monday, 2:15. 18

Mapping the binding epitopes for cadherin-like receptor (BT-R1) on *Bacillus thuringiensis* Cry1Aa toxin

Xinyan Liu, Donald H. Dean

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CAD-D, a truncated fragment (CR11 and 12) of Bt-R1a, the cadherin-like receptor from *Manduca sexta* for *Bacillus thuringiensis* Cry1A toxins, was expressed and purified as a soluble MBP (maltose binding protein) fusion protein. Binding affinity of Cry1Aa to CAD-D measured by real time SPR was at 10nM level. CAD-D binding epitopes on Cry1Aa toxin were mapped by alanine scanning mutagenesis. Designing of the area targeted for mutagenesis was based on structural information derived from topology prediction and computational docking of the toxin with the receptor. Loop 2 residues in Domain II and three clusters of surface residues in Domains II and III were demonstrated to be involved in binding to CAD-D. The interaction surface was defined by the loss of binding for mutants on the predicted face of the toxin and no effects on another set of substitutions located on the opposite face of domain III.

Contributed paper. Monday, 2:30. 19

A detergent-like mode of action of the Bt toxin Cyt1A

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To distinguish between the "pore-forming" and "detergent" hypotheses of the Cyt1A's mode of action we investigated whether in the presence of lipid Cyt1A self-assembles into stoichiometric oligomers, which are characteristic of pores, or aggregates into non-stoichiometric complexes, which would support the detergent-like model. Sodium dodecyl sulfate polyacrylamide gel electrophoresis revealed that in the presence of lipid Cyt1A forms protein aggregates with a broad range of molecular weights. Cyt1A tryptophan fluorescence in the presence of lipid exhibited a decrease in

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 Cyt1A 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 Cyt1A.

STU Contributed paper. Monday, 2:45. 20

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 Quievy, Jean-Louis Schwartz and Raynald Laprade

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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 membrane vesicles 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-phenanthroline 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. 21

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

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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 toxin concentration, 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. 22

Mode of action of *Bacillus thuringiensis* insecticidal toxin Cry9Ca: Effect of the physico-chemical microenvironment on pore formation in *Manduca sexta* intestinal membranes

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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. 23

Directed mutagenesis of conserved aromatic residues in helix 7 critical for larvicidal activity of the *Bacillus thuringiensis* Cry4Ba toxin

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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 mutagenic analysis showed that only replacements with an aromatic residue, Y249F or Y249W and F264Y or F264W, still retained the high level in 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. 24

Mutagenic analysis of the transmembrane helix 5 of the *Bacillus thuringiensis* Cry4Ba toxin reveals a crucial role in larvicidal activity for Asn-183

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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 hydrophobic 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 (Div. of Microbial Control). Monday, 4:20-6:20

Use of pathogens against incursion pests

Symposium. Monday, 4:20. 25

Eradication of incursive Lepidopteran pests with Foray

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Foray 48B is a Btk HD-1 based product which is widely used in the control of lepidopteran pests in forests. Control programmes are usually under the control of government agencies and target native or established exotic pests. These programmes can involve aerial sprays over towns and cities and the health and environmental impacts have been widely researched and documented. In the last decade, Foray has been used by the New Zealand Government not to control, but to eradicate incursive exotic lepidopteran pests. These eradication programmes require routine aerial and ground applications of Foray over urban areas and involve considerable social, political and mental angst in the community which significantly interacts with the desired efficacy goals. "Operation Ever Green" was a mid-90's NZ Government programme which prided itself on being the first ever successful eradication of an exotic forest pest established in an urban area. The results of the 2002-04 "Painted Apple Moth Programme" are still being evaluated but look positive. The health, environmental and efficacy outcomes of these programmes, as well as others from around the world, have been reported in both government publications and the scientific literature. Valent BioSciences strictly limits its involvement in these urban spray programmes but has provided assistance to the government agencies involved in relation to efficacy and aircraft set-up, regulatory, public health and scientific review. These aspects will be discussed in this paper.

Symposium. Monday, 4:44. 26

Assessing short-term human health effects of *Bacillus thuringiensis* applied during insect control programs

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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.

Symposium. Monday, 5:08. 27

Use of pathogens against incursion pests in New Zealand

Travis R. Glare[1] and Ian R. Gear[2]

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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 anartoides*, 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. Hajek[1], James R. Reilly[1], Thomas Dubois[1], Michael Smith[2], Leah Bauer[3] and Zengzhi Li[4]

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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, 5:56. 29

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

Rosalind R. James

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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 market. The beekeeping market is small, and some of the infrastructure necessary to produce a product is currently lacking, so investment opportunities are limited.

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

VIRUSES 1

Contributed paper. Monday, 4:20. 30

Pathogen diversity and the efficacy of virus insecticides

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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 I. Graham[1], Shujing Rao[2], Steven M. Sait[3], Robert D. Possee[1], Peter P. C. Mertens[2] and Rosemary S. Hails[1]

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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 (*Phobocampe 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

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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 cathepsin. 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 abrogates 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 while 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)

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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 *Bam*HI, *Eco*RI, *Pst*I and *Xho*I. 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 ST_{50}) 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* Boisd. (Lepidoptera:Noctuidae) by an optical brightener

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Previous studies have shown that certain optical brightners 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* Boisd. (Lepidoptera: Noctuidae) larvae by an optical brightner 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 Lee[1,2], Jenny S. Cory[3], Kenneth Wilson[2], David Raubenheimer[1] and Stephen J. Simpson[1]

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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 a nucleopolyhedrovirus, under 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

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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 ecdysone titers might control both behaviors. Inactivation of ecdysone 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 marginiventris* (Cresson)

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The recombinant baculovirus AcMLF9.ScathL expresses a basement membrane-degrading protease (ScathL) 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 marginiventris* (Cresson) that parasitizes infected larvae. Larvae of *Heliothis 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 AcMLF9.ScathL was lower than from those infected with wild-type virus. There were no significant differences between AcMLF9.ScathL 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 ScathL-induced melanization of host larvae. These results indicate that AcMLF9.ScathL 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 lymantriae* and *Vairimorpha* sp. parasitizing *Lymantria dispar* larvae: The importance of timing for successful establishment and horizontal transmission of infection

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We studied competition between *Nosema lymantriae* 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

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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

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Although microsporidia were first discovered to infect 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.

Contributed paper. Monday, 5:05. 41

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

Susan Bjornson

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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 effective for controlling aphids under such circumstances. To complicate issues, lady beetles are known to host to several parasites and pathogens, including microsporidia and these may affect their ability 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 used for biological control. 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 *Paranosema locustae* represent two different developmental sequences of the same species?

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Locusta migratoria (Acrididae: 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. capito*, are type hosts of two microsporidia, *Paranosema locustae* and *Johenrea locustae*, respectively. Adipose tissue is the main site of infection of both pathogens. Morphologically, *Paranosema* and *Johenrea* are remarkably different. *Paranosema* is diplokaryotic, apansporoblastic, and disporoblastic, and causes a diffuse infection. *Johenrea* is haplokaryotic, forms sporophorous vesicles, has larger spores, and develops xenomas. *Johenrea* spores isolated from *L. m. capito* 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 *Paranosema*. Infection of *L. m. migratorioides* with spores produced in *T. pallidipennis* resulted in partial reproduction of "*Johenrea*" characters, but also the formation of "*Paranosema*-like" spores. Isolation of DNA from *J. locustae*-infected *L. m. capito* 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*.

STU Contributed paper. Monday, 5:35. 43

Microsporidian parasites in freshwater snails

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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.

STU Contributed paper. Monday, 5:50. 44

PfPuf2, a translational repressor, regulates sexual development in the malaria parasite *Plasmodium falciparum*

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Sexual development in malaria parasites is an obligatory process for the transmission of the disease through mosquito vectors. Understanding of the molecular mechanism of this biological process may offer novel venues for the control of the disease. Recently, we have identified members of the Puf RNA binding protein (RBP) family in *Plasmodium falciparum*. Research with genetically amenable organisms such as *Drosophila* has shown that the RNA-binding domains of these proteins are conserved both in structure, with eight imperfect tandem repeats, and in function, to maintain germline stem cells through translational repression of the target RNAs. To investigate the role of Puf proteins in *P. falciparum* development, we have characterized the expression, RNA-binding activity and biological function of *PfPuf2*. Using Northern and western blots, we showed that PfPuf2 was preferentially expressed in sexual stages. Using the yeast three-hybrid system, which studies protein-RNA interactions, we demonstrated that PfPuf2 RNA-binding domain had specific RNA-binding activity to the conserved target RNA sequence of the Puf family. This suggests that PfPuf2 utilizes a similar mechanism of translational control of its target genes. To functionally characterize PfPuf2 in parasite sexual development, we transfected the parasite in order to achieve targeted gene disruption of *PfPuf2*. Parasites were selected for resistance to pyrimethamine and clones with stable disruption of the *PfPuf2* locus were obtained. This allowed the expression of truncated PfPuf2 RNA-binding domain, which eliminates the RNA-binding activity of the protein. Disruption of *PfPuf2* in these parasite lines was confirmed by integration-specific polymerase chain reaction (PCR), genomic Southern blot, and reverse transcriptase-PCR. Phenotypic analysis showed that *PfPuf2* disruption did not affect asexual growth of the parasite, but promoted the formation of gametocytes. This is consistent with the ancestral function of this protein family in promoting proliferation and suppressing differentiation. The effect of *PfPuf2* disruption on parasite sporogonic development in vector mosquitoes is under investigation. The conservation of this protein family in other apicomplexan parasites may implicate a similar function governing sexual development.

STU Contributed paper. Monday, 6:05. 45

Analysis of *Gregarines niphandrodes* mitochondria

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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. niphandrodes* 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 niphandrodes* 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 niphandrodes*.

Workshop (Div. of Viruses). Monday, 7:45-8:45

Microarray technology, genomics and proteomics in entomopathogen research

Workshop paper. Monday, 7:45. 46

Microarray technology, genomics and proteomics in entomopathogen research

Peter J Krell[1], David A Theilmann[2,4], Luke Masson[3], Manuella van Munster[3] Dan-Hui Yang[1], Ilse Huijskens[2,4] and Martin A Erlandson[5]

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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 acquisition/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.

Workshop (Div. of Microsporidia). Monday, 7:45-8:45

Tranmission and ecology of Microsporidia: A broad spectrum of possibilities

Workshop paper. Monday, 7:45. 47

Experimental study of transmission of Microsporidia from blood-sucking mosquitoes of Siberia, Russia

Anastasia Simakova[1] and Tamara Pankova[2]

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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 *Anopheles* and *Aedes* mosquitoes were used for per oral infections of nauplial, copepodite stages and adult crustaceans (16 species of Cyclopoida, 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 refrigerator or at room temperature (from 0 to 25ºC). Experiments were conducted with spring, summer, autumn and winter generations of crustaceans in laboratory conditions (room temperature from 14 to 28ºC) 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 octosporic genera from blood-sucking mosquitoes to the same or another species of mosquitoes, lower crustaceans and insects.

Workshop paper. Monday, 8:00. 48

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

Tamara Pankova[1] and Anastasia Simakova[2]

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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 accumulation of infection over the season. On the whole studying period several outbreaks of microsporidioses were registered. In 1976 *Aedes* mosquitoes larvae were almost totally infected by *Amblyospora* species with fatal consequences. In following years rates of infections decrease. In *Anopheles* populations with *Parathelohania* species infections in the 1980th also several epizooties were registered. However, during last years we registered approximately equal levels 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%.

Workshop paper. Monday, 8:15. 49

Exploring horizontal field transmission of Microsporidia

Vincent D'Amico[1], Leellen Solter[2], Milan Zubrik[3], Michael McManus[4], and Gernot Hoch[5]

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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 Levishte, 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.

STU Workshop paper. Monday, 8:30. 50

Transmission of *Nosema fumiferanae* in spruce budworm populations

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A sublethal microsporidian, *Nosema fumiferanae*, may aid in driving spruce budworm population oscillations. Within a generation, horizontal transmission is the primary mode responsible for increasing *Nosema* prevalence. We infected laboratory larvae to parameterize the rate of transmission, and in the greenhouse we utilized vertically infected individuals to explore the ecology of horizontal transmission. In the laboratory, horizontal transmission was affected by temperature ($F_{1,37} = 7.49$, $p = 0.009$) and was reduced at higher temperatures. Larval density did not affect horizontal transmission in the laboratory ($F_{2,24} = 2.61$, $p = 0.051$) or in the greenhouse ($F_{4,18} = 2.52$, $p = 0.077$). Horizontal transmission was significantly affected by initial infected density ($F_{4,18} = 7.66$, $p < 0.001$). Experiments are in progress to further parameterize and study horizontal transmission. Additionally, in mid 2005, we will monitor a local budworm population for *Nosema* prevalence throughout the larval stage as an indicator for horizontal transmission. Vertical transmission of *Nosema* determines the initial density of infected larvae in the next generation. Infected adults eclose later than uninfected adults, which may play a role in passing infection to the next generation. We will examine the effect of *Nosema* spore density.

Workshop (Div. of Fungi). Monday, 7:30-9:30

Systematics and ecology of Entomophthorales

Workshop paper. Monday, 7:30. 51

Systematics of the arthropod-pathogenic Entomophthorales

Siegfried Keller

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225 species of arthropod-pathogenic Entomophthorales are described. They are attributed to four families, however, the large majority belongs to the families Entomophthoraceae (196 species including 38 species of *Tarichium*) and Neozygitaceae (18 species). Morphological studies and a compilation of the literature data led to the description of three subfamilies of the Entomophthoraceae and to the description of a new genus in the family Neozygitaceae. Further, "weak" points in the systematics were localised and research gaps addressed.

Workshop paper. Monday, 8:30. 52

Bizarre interactions and endgames: Entomopathogenic fungi and their arthropod hosts

Helen Roy[1], Don Steinkraus[2], Jorgen Eilenberg[3], Ann Hajek[4] and Judith Pell[5]

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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.

Workshop paper. Monday, 8:50. 53

Ecology of Entomophthorales: A European perspective

Jörgen Eilenberg[1], Stanislaw Bałazy[2], Jürg Enkerli[3], Anselme Fournier[3], Annette Bruun Jensen[1], Siegfried Keller[3], Charlotte Nielsen[1], Cezary Tkaczuk[4], Franco Widmer[3]

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The European COST Action 842 on Entomophthorales had as one aim to reveal novel information about the ecology of Entomophthorales. This was done by: 1) Workshops on practical sampling and diagnosis of Entomophthorales 2) Workshop discussions on methodologies for prevalence assessment 3) Initiation of new research programmes 4) Joint actions between scientists from different countries With respect to 1) collection trips were performed in Switzerland and Poland, for example in the nature reservation of Białowieza with high biodiversity of Entomophthorales. Concerning 2) we tended to develop consensus about terms like "prevalence" and the practical implications of these for studies on Entomophthorales. With respect to 3) new Danish projects on the genetic composition of host and pathogen in an aphid-*Pandora neoaphidis* system as well as the winter survival of the fungus will be presented. In addition, we will report on a Swiss project on the development of cultivation independent genetic tools for the study of *P. neoaphidis* in the environment. Finally, the action supported the possibilities for joint projects between European laboratories 4). One example of this is a co-operation between a Swiss and a Danish team to explore PCR techniques for cultivation independent tracking of Entomophthoralean species in soil samples.

Workshop paper. Monday, 9:10. 54

Ecological studies underpinning the development of conservation biological control with *Pandora neoaphidis* in UK

J.K. Pell[1], S. Ekesi[2], A.M. Tymon [3] and P.A. Shah[4]

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Reforms to the Common Agricultural Policy in Europe encourage farming practices which protect the environment and maintain biodiversity in agroecosystems. This includes the planting of flower and seed rich field margins supporting insects and birds. With small adjustments these margins could also maintain populations of beneficial organisms such as entomopathogenic fungi, thereby contributing to pest management. We hypothesised that *Pandora neoaphidis*, a common aphid pathogenic fungus in temperate field crops, could be exploited in this way. Fundamental ecological studies on host range, dispersal and transmission were required to underpin this strategy. Field margin plants with potential as reservoirs of *P. neoaphidis* were identified, e.g. Yorkshire fog (*Holcus lanatus*), legumes such as bird's-foot trefoil (*Lotus corniculatus*) and stinging nettles (*Urtica dioica*). Cross transmission and dispersal of the fungus between aphid species occuring in margins and crops and positive interactions with other natural enemies were demonstrated. Pest and non-pest aphids varied in their susceptibility to *P. neoaphidis* but there was no biological or molecular relationship between original host of an isolate and host range suggesting that free movement of *P. neoaphidis* between different aphid hosts was possible. These data underpin the exploitation of field margins as reservoirs of *P. neoaphidis* for aphid control.

TUESDAY – 9 August

SYMPOSIUM (Cross-Divisional). Tuesday, 8:00-10:00

Transmission of invertebrate pathogens

Symposium. Tuesday, 8:00. 55

The evolution of virulence and transmission of disease

Philip Agnew

Génétique et Evolution des Maladies Infectieuses, CNRS / IRD - UMR 2724, 911 Avenue Agropolis (bp 64501), 34394 Montpellier Cedex 05, France.

A pathogen's virulence is an important trait for anyone concerned with pathogens or the host populations they attack. It is also a trait often related to a pathogen's transmission success. The ability to predict or eventually manage the evolution of a pathogen's virulence is a highly desirable goal. Many theoretical models have been developed with this in mind. The aim of my talk will be to outline how these models are constructed, the trade-offs involved, and how predictions are made. Although much of the data required for such models is generated during studies of invertebrate pathology, I will

highlight 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

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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 epizootics 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 *Neozygites fresenii*.

Symposium. Tuesday, 8:48. 57

Consideration of vertically transmitted microsporidia for biological control

Leellen F. Solter

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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 *Vairimorpha* 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

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The most common occluded viruses of mosquitoes are baculoviruses (nucleopolyhedroviruses, NPV) and cypoviruses (CPV). Mosquito NPV's have a circular, double-stranded DNA genome packaged into rod-shaped enveloped capsids embedded in a protein matrix. Mosquito cypoviruses 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

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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, monogyny (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 polygyne 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 monogyne field colonies of *S. invicta* have been reported from Louisiana and Argentina. Alate queens with the monogyne genotype have a greater dispersal capability than polygyne alates and could potentially facilitate the spread of the pathogen. Demise of infected monogyne colonies can be twice as fast as in polygyne colonies and favors the pathogen's persistence in polygyne 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 (Division of Fungi). Tuesday, 8:00-10:00

Emerging genomics of fungal entomopathogens

Symposium. Tuesday, 8:00. 60

Generation of a robust EST dataset for the entomopathogenic fungus *Beauveria bassiana*

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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 microcycle 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.

Symposium. Tuesday, 8:30. 61

Developmental and transcriptional responses to host and non host cuticles by the specific locust pathogen *Metarhizium anisopliae* var. *acridum*

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Transcript patterns elicited in response to hosts can reveal the mechanisms involved in pathogenicity. These patterns could be fashioned by recognition of host specific topographical features or by chemical components displayed or released by the host. We investigated this in the specific locust pathogen *Metarhizium anisopliae* var. *acridum*. Only host (*Schistocerca gregaria*) cuticle stimulated the full developmental program of germination and differentiation of infection structures (appressoria). Cuticle from beetles (*Leptinotarsa decimlineata*) repressed germination while cuticle from hemipteran bugs (*Magicicada septendecim*) allowed germination but only very low levels of differentiation. Using organic solvents to extract insects we identified a polar fraction from locusts that allowed appressorial formation against a flat hydrophobic surface. Microarray analysis determined that of 483 differentially regulated genes 97% were upregulated by the polar fraction. These included genes involved in metabolism, utilization of host cuticle components, cell survival and detoxification, and signal transduction. Surprisingly, very similar transcript profiles were observed on locust and beetle extracts. However, the beetle extract cluster was enriched in genes for detoxification and redox processes while the locust extract upregulated more genes for cell division and accumulation of cell mass. In addition, several signal transduction genes previously implicated in pathogenicity in plant pathogens were only upregulated in response to locust extract implying similarities in the regulatory circuitry of these pathogens with very different hosts.

Symposium. Tuesday, 9:00. 62

Linking ESTs to gene function and secondary metabolite discovery in *Metarhizium anisopliae*

Alice C.L. Churchill

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Although entomopathogenic fungi have great potential as insect biocontrol agents, there is an incomplete understanding of the genetic factors that make them effective. My collaborators and I have focused our efforts on understanding the roles of toxins as virulence factors in fungal-insect interactions. Fungi generally make three main classes of secondary metabolites: nonribosomal peptides, polyketides, and terpenes, as well as hybrid molecules consisting of these and other moieties. We and others have cloned secondary metabolite biosynthetic genes from fungal EST libraries or by using degenerate primers to clone fragments of nonribosomal peptide synthetase (NRPS) and polyketide synthase genes. As a first step toward determining the function of genes predicted to synthesize secondary metabolite toxins and to identify the roles of such toxins in the biology of *Metarhizium anisopliae*, we developed efficient *Agrobacterium tumefaciens*-mediated transformation and gene disruption methodologies for the fungus. In this talk, I will describe our analyses of DNA polymorphisms and gene expression for several secondary metabolite genes and our gene disruption studies of a single NRPS correlated with production of the cyclic peptide toxins called destruxins. Additionally, I will discuss our discovery of a group of polyketide mycotoxins, not previously reported from *Metarhizium*, that were first detected in a genetically modified *M. anisopliae* transformant.

Symposium. Tuesday, 9:30. 63

Sense and sensibility in the Genomic Age

Richard A. Humber

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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 isolate should be chosen? Once one of these fungi is 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.

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

NEMATODES AND SYMBIOTIC BACTERIA

Contributed paper. Tuesday, 8:00. 64

Insecticidal toxins from *Photorhabdus* bacteria

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

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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 (Tc's) 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 mosquitoe 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

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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

level of potency. We also demonstrate that a single set of potentiators can potentiate several Class A proteins.

Contributed paper. Tuesday, 8:30. 66

Novel toxin complex constructions

Timothy Hey, Charles Cai, Aaron Woosley, Stephanie Burton, Joel Sheets, Brian Waldman, Haley Moon, Tom Meade and Don Merlo

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The Toxin Complex proteins from *Photorhabdus* 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. Lee[1], Svetla McPhie[2], Alison Rodger[3], David I. Roper[2], Janey Henderson[5], Martin Sergeant[1] and J.Alun W. Morgan[1]

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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* pMFI296) toxins that are able to kill the insects *Pieris brassicae*, *Plutella xylostella*, *Heliothis verescens* 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 monodispersed, 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 apoptosis

Fabienne Vigneux[1], Alain Givaudan[1], Pierre Alain Girard[1], Carlos Ribeiro[1], Stephen Baghdiguian[2] and Michel Brehélin[1]

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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 species 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 nucleotidic 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.

Contributed paper. Tuesday, 9:15. 69

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

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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 receptacle 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 receptacle, 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*

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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 LT_{90} 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. 71

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

Xinyi Li[1], Richard S. Cowles[2], Elizabeth Cowles[3], Randy Gaugler[4], A. Daniel Jones[5] and Diana L. Cox-Foster[1]

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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 immuno suppression is dose dependent. Also, multiple injections of the SPCs protected *H. bacteriophora* better in the oriental beetle larvae. In nondenatured 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. 72

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

Sandhya Boyapalle[1], Randy Beckett[2], W. Allen Miller[2], Bryony C. Bonning[1]

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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, *Homalodisca 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. 73

Baculovirus expression of *Rhopalosiphum padi* virus (Dicistroviridae)

Sandhya Boyapalle[1], Randy Beckett[2], W. Allen Miller[2], Bryony C. Bonning[1]

[1]Departments of Entomology and [2]Plant 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 AcRhPV6. Expression of the RhPV genome in Sf21 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 Sf21 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 Sf21 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.

STU Contributed paper. Tuesday, 8:30. 74

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

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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 virginoparae 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.

Contributed paper. Tuesday, 8:45. 75

Comparative viral RNA loads in deformed wing virus infected *Apis mellifera* L. and its ectoparasite *Varroa destructor*

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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, however bees displaying wing deformities showed higher values. In drone bee propupae, the DWV RNA loads were higher in those parasitized by more than two mother mites. In varroa, the DWV RNA yields may exceed 10^8 equivalent DWV RNA copies per mite.

Contributed paper. Tuesday, 9:00. 76

Analysis of the poly(A) polymerase encoded by the entomopoxvirus, AMEV

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The eukaryotic enzyme, poly(A) polymerase catalyzes addition of adenosine (A) residues to the 3' end of mRNA. All viruses of the Poxviridae family, including the entomopoxvirus from *Amsacta moorei*, (AMEV) transcribe mRNAs in the cytoplasm from the viral DNA template. The viral cytoplasmic transcriptional apparatus includes a poly(A) polymerase. The poly(A) polymerase from vaccinia virus (VV), is a heterodimer consisting of a large subunit (E1) and a small subunit (J3). The multifunctional J3 subunit confers processivity to the poly(A) addition initiated by the E1 subunit resulting in longer poly(A) tails. J3 also possesses 2'O-methyltransferase activity and functions as a positive transcription elongation factor. Unlike VV, AMEV encodes *two* small subunits, AMEV060 and AMEV115 both with similarity to VV J3. AMEV060, like VV J3 is expressed early, prior to DNA replication, whereas AMEV115 is expressed as a late gene. We show that AMEV060, like J3 possesses 2'O-methyltransferase activity. Surprisingly, AMEV060 can be deleted without effects on virus growth in cell culture. Experiments are underway to attempt deletion of AMEV115. The effects of deleting the small subunit genes on mRNA synthesis and mRNA poly(A) addition is being evaluated. Interactions of each AMEV small subunit with the large subunit are being explored.

Contributed paper. Tuesday, 9:15. 77

Virus tropisms is controlled by insect parvovirus promoters

Peter Tijssen, Jozsef Szelei, Mohamed El-Far and Gilles Fédière

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MlDNV and GmDNV are two closely related densoviruses. They have over 90% of sequence identity, same genome organisation, and use a similar expression strategy, but have different host preferences. While GmDNV is restricted to its *Galleria mellonella* host, MlDNV infects a broad range of lepidopteran species, in addition to *Mythimna loreyi*. *In vitro* screening on four insect cell lines showed similar behaviour for their tropism when infectious clones from GmDNV and from MlDNV were used. MlDNV infected several different types of cells whereas GmDNV was only infectious to LD-652 cells. We have used the two infectious clones to create a number of chimeras between the two viruses by swapping homologous restriction fragments that cover the whole genome parts, ITRs, NS- and VP-cassette. Transfection experiments showed that neither of the coding sequences was responsible for the change in tropism. The transfer of MlDNV-VP promoter into the GmDNV background broadened the host cell specificity. The activities of GmDNV- and MlDNV-VP promoters were further characterized in a CAT reporter gene based transient expression system in the different cell lines.

STU Contributed paper. Tuesday, 9:30. 78

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

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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.

STU Contributed paper. Tuesday, 9:45. 79

Characterisation of *Cydia pomonella* granulovirus metalloproteinase

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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 (Division of Viruses). Tuesday, 10:20-12:20

Polydnaviruses and Ascoviruses

Symposium. Tuesday, 10:20. 80

A polydnavirus paradox: Cophylogeny and mosiac genomes

James Whitfield

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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 bracoviruses 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 cophylogenetic pattern extends throughout the bracovirus-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 identifiying 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

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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-Wen Cheng

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Ascoviruses are transmitted to lepidopteran larvae by parasitoids mechanically during oviposition. There are four official ascovirus species accepted by ICTV. The *Diadromus 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 *Heliothis virescens 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 entomopoxvirus in cytoplasm with ascovirus as a link.

Symposium. Tuesday, 11:50. 83

The biology of polydnaviruses and their interactions with insect hosts

Nancy Beckage

Departments of Entomology and Cell Biology and Neuroscience, University of California, Riverside, USA

Parasitoid polydnaviruses (PDVs) have potent biological effects on insect hosts. These viruses induce disruption of host immune function and development. In highly co-evolved host-parasitoid systems, expression of PDV genes in host hemocytes induces their apoptosis and prevents encapsulation of the parasitoid egg. In refractory hosts, expression of PDV genes is suppressed, and the parasitoid is avidly encapsulated. In tobacco hornworm larvae parasitized by *Cotesia congregata*, production of six CcPDV encoded transcripts begins as early as 30 min post-oviposition in host fat body and hemocytes. Antibodies to the virally encoded protein CrV1 bind to the hemocyte surface as well as to foci in the cytoplasm of the apoptotic cells. Chelonine wasp PDV genes are expressed in synchrony with appearance of symptoms of developmental derangements in the host caterpillar. Thus, the endocrine as well as the immune system serves as a target for PDV activity. Polydnavirus receptors have yet to be isolated in host endocrine glands but likely exist. The "essence" of a parasitoid is that its presence causes developmental arrest of the host in the larval or pupal stage prior to adult eclosion. The PDVs of many parasitoids have been shown to induce this type of arrest when injected into nonparasitized "surrogate" hosts.

CONTRIBUTED PAPERS. Tuesday, 10:20-11:35

ALGAE, OTHER

Contributed paper. Tuesday, 10:20. 84

Women pioneers of invertebrate cell culture

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Richard Goldschmidt started insect tissue culture at Yale University in 1915. One night he was arrested, accused of spying, and interned in Georgia. His lady coworker was also arrested but Goldschmidt never mentioned her in his papers. She was actually the first woman pioneer of invertebrate cell culture. Following the 1935 work of William Trager, in 1945 insect culture media were greatly improved by Silver-Wyatt in Canada. In 1962 at the 1st International Colloquium on Invertebrate Tissue Culture in Montpellier a large number of pioneer women presented their work: C. Sengel, Nadine Plus, Annie Ohanessian, Amargier, M. F. Maury, S. Chastang, Rosine Chandebois (France), A. M. Leloup (Belgium), R. Grigorova (Bulgaria) and several others. At the 2nd Colloquium in Tremezzo, Italy in 1967 Silvania Dolfini, Carlotta Halfer (Italy), Mary Pudney (U.K.), Imogene Schneider (USA) and Helena Libikova (Slovakia) contributed important papers. Absent were Medvedeva (Ukaine), not permitted to attend the conference abroad, and Usha Pant (Pune, India). Afterwards in the United States Sonya Buckley, Marion A. Brooks, Marcia Loeb, Elizabeth Gateff and Imogene Schneider became well known for their important contributions, soon followed by Cynthia L. Goodman and Ulrike G. Munderloh.

Contributed paper. Tuesday, 10:35. 85

Genomics approaches to insect-pathogen relationships in the spruce budworm, *Choristoneura fumiferana*

Qili Feng[1], Tim Ladd[1], Sichun Zheng[1, 2], Lan Li[1], Dayu Zhang[1,2], Debora Buhlers[1], Peter J. Krell[2], Basil M. Arif[1], Arthur Retnakaran[1]

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Genomics approaches, such as expressed sequence tags (ESTs) and microarrays, present a unique opportunity to study interaction 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.

Contributed paper. Tuesday, 10:50. 86

Development and pathway of infection of the entomopathogenic alga *Helicosporidium* (Chlorophyta: Trebouxiophyceae)

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This study examined the morphogenesis and growth dynamics of different cell phenotypes of *Helicosporidium* spp., a unicellular, non-chlorophytic 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 autosporulation. 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

***Helicosporidium* sp. infection in mosquito larvae**

Tracy M. Conklin[1], Verena-Ulrike Bläske[1], James J. Becnel[2], and Drion G. Boucias[1]

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Members of the genus *Helicosporidium* 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 *Helicosporidium* 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

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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 Nam[1], In-Pyo Hong[1], Jae-Sam Hwang[1], Seung-Beom Hong[2], Sang-Duk Ji[1], Seok-Woo Kang[1] and Myung-Sae Han[3]

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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. tenuipes* 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

stromata nor development of secondary spores was recognizable, while the number, shape and colour of stromata varied with insect hosts and weather conditions. ITS 1, ITS 2 and 5.8 rDNA 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. tenuipes* 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.

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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 polymorphous bands. The dendrogram showed that the isolates of *B. bassiana* from 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.

Poster / Fungi. F-3.

Characterization of *Beauveria bassiana* isolates based on ITS and TEF sequences

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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-ITS2 including 595 nucleotids , demonstrated that 0.16-1.77% sequence variation existed among ten isolates. Three distinct groups were determined using UPGMA clustering which slightly correlated with origin of isolates (insect host or soil). Protein coding gene, *tef-1α* sequence alignment indicated that there is a 1.8% sequence variation among isolates. Sequencing *tef -1α* 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 decemineata*.

Poster / Fungi. F-4.

Approaches to testing a biological hypothesis that host flight dominates transmission of aphid-pathogenic fungi among aphid populations

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Entomophthorales-caused epizootics are of general importance in natural control of aphids. Transmission of aphid-pathogenic fungi is most likely to be associated with their host dispersal by actively hovering over crops or passively flying with winds for long distances. This hypothesis can be proved with either an evidence that fungal pathogens are present in migratory alates trapped from air >30 m above the ground or an evidence that infected alates enable to initiate new colonies after a distant flight during a latent period and their mycoses can be transmitted to offspring. Two experimental approaches were developed to examine the hypothesis. Firstly, migratory alates were trapped from air onto potted plants placed on tower buildings, collected at daily intervals and individually reared in laboratory for up to 7 days. Examining all alates mycosed within this period led to estimating the frequencies of fungal pathogens borne by the air-trapped alates. Secondly, inoculated alates were individually flown in a computer-monitoring flight mill system to estimate their flight capability and then reared to evaluate their potential for reproduction and mycosis transmission in offspring colonies. Data from such experiments performed in the past few years were presented, including the results based on >10,000 air-trapped alates in several provinces of China and simulation flight experiments with several hundreds of inoculated *Sitobion avenae* and *Myzus persicae* alates in laboratory.

Poster / Fungi. F-5.

Effects of entomopathogenic fungus *Paecilomyces fumorososeus* to common white *Pieris rapae crucivora*

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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. Conidial suspension ($1.0x10^7$ 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.

Poster / Fungi. F-6.

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

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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: LC_{50} = $1x10^7$ conidias ml^{-1}, TL_{50} = 7 days; adults: LC_{50} = $1,3x10^7$ conidias ml^{-1} and TL_{50} = 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 prospective for usage in Integrate Pest Management strategies of *A. tuberculatus*.

STU Poster / Fungi. F-7.

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

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The glassy-winged sharpshooter (GWSS), *Homalodisca coagulata* (Homoptera: Cicadellidae), is a highly polyphagous xylophage 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-occuring entomopathogens. Three possible organisms were found, the most prevalent of which was a novel species of *Hirsutella*, 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 *Hirsutella* 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

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Mass-produced biocontrol agents Mycotal and Vertalec have a high virulence against whitefly and aphid, respectively. Strain B-2 has a higher colonizing ability on a cucumber leaf as an epiphyte (Koike et al. 2004). In this study, we performed protoplast fusion experiment among 3 strains of *Verticillium lecanii* to breed useful new strains those had useful characteristics as biological control agents. *Nit* mutant was applied as a genetic marker, in order to visualize a colony that had undergone fusion. Complementary parental protoplasts were mixed, and fused by PEG method. 126, 44 and 4 hybrid strains were detected from combination of Vertalec×Mycotal, B-2×Mycotal and B-2×Vertalec, respectively. Furthermore we observed vigorous and vertical growth colonies among them, and measured colony growth speed and the amount of the spore production. Some of them showed rapid growth when compared to parental isolates. It indicated that such vigorous growth might be an important factor to improve the virulence. It also showed that the protoplast fusion could be an effective methods to breeding the entomopathogenic fungi. We also report screening of virulence to aphid and whitefly.

Poster / Fungi. F-9.

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

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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.

STU Poster / Fungi. F-10.

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

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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 melonella* 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.

Poster / Fungi. F-11.

Comparison of *Galleria* baiting and soil plating methods for isolating soilborne pathogens from the habitats of glassy-winged sharpshooter, *Homalodisca coagulata* (Homoptera: Cicadellidae), in California

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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.

Poster / Fungi. F-12.

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

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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. anisopliae* var. *anisopliae* ARSEF 2575, ARSEF 3609, ARSEF 5749 and *M. anisopliae* 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. 10^5 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 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.

Poster / Fungi. F-13.

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

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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 groups of six in a suspension of 1 x 10^7 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 Flucker's high calcium cricket feed) and sagebrush 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 instar nymphs; and two trials were conducted with adults. ARSEF 2575, with a LT_{50} 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 LT_{50} 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.

Poster / Fungi. F-14.

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

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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. anisopliae* 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.

STU Poster / Fungi. F-15.

Are 'stressed-out' wireworms more susceptible to the biocontrol agent *Metarhizium anisopliae*?

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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 effects 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.*

Poster / Fungi. F-16.

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

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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 Ulilid fly *Tetanops myopaeformis*. These fungi can be used in the form of a granular formulation applied at planting, and/or as an aqueous, band-over-row spray of conidia to the bases of the plants just before peak fly oviposition. Recently we have evaluated another strain, F52, which holds more promise via greater infectivity, as well as better commercial attributes. Laboratory studies of soil abiotic and biotic factors affecting persistence and efficacy with F52 and MA1200, have yielded considerable information about the constraints and challenges in using *Metarhizium* in sugar beets, and overall potential as a successful biocontrol agent of soil dwelling insects. For example, seed coat fungicides, toxic *in vitro*, do not affect adjacent *Metarhizium* granules in soil. Infectivity of conidia is generally proportional to soil moisture, but soil type and moisture interact to complicate this relationship. The fungus is not efficacious by itself in the face of high insect pressure but needs integration with other tools. (Research was supported in part by CSREES NRI Grant #0189370.)

Poster / Fungi. F-17.

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

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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 developing sugarbeet 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-18.

Influence of plant rhizosphere on the abundance of entomopathogenic fungi

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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* L.) and lupine (*Lupinus albus* L.) 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 dodine-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.

Poster / Fungi. F-19.

Colonization of sugarbeet roots by entomopathogenic fungi

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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 sugarbeet 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 pTEFEGFP, 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.

Poster / Fungi. F-20.

Coffee endophytes pathogenic to the coffee berry borer

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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 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 *Meira* 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 at 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-21.

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

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Among fungi with no known sexual stage or are predominantly 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 hyphae fuse 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-22.

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

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The contents of hydrophobin-like or formic-acid-extractrable (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 thermotolerance 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 consisted of 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*

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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-alkenoyl-3, 4-epoxy-2-pyrrolidinone moiety attached to a substituted pentaene unit that isomerizes readily, especially upon exposure to light.

STU Poster / Fungi. F-24.

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

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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 cue 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 *Periplanata 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

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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 polyacrylamide gel electrophoresis we had identified that *Beauveria bassiana* strains ALG produces PR1 and PLB2 proteinases. 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.

Poster / Fungi. F-26.

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

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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*

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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 Vip3Ab1 than that of Vip3Aa proteins. 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-DCCEEDCCEEDCCEEDCCEE-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.

STU Poster / Bacteria. B-2.

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

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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*, *vip3Ae2*, 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.

Poster / Bacteria. B-3.

Novel insecticidal proteins secreted by *Bacillus thuringiensis*

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A new family of insecticidal proteins secreted by *Bacillus thuringiensis* was discovered. The ability of *B.t.* strains to produce a variety of insecticidal parasporal crystals (δ-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.

Poster / Bacteria. B-4.

Antibacterial activity of *Bacillus thuringiensis* strains

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The antibacterial activity 67 of *Bacillus thuringiensis (Bt)* strains of 43 subspecies against *Micrococcus lysodeikticus, 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* ssp. *thuringiensis, alesti, kurstaki, sumiyoshiensis, fukuokaensis, galleriae, entomocidus, darmstadiensis, dakota, shandongiensis, neoleonensis, mexicanensis, cameroun, 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* ssp. *kurstaki* and ssp. *galleriae*) showed sensitivity to their own bacteriocins.

Poster / Bacteria. B-5.

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

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Two novel *cry* genes, *cry30C* and *s2orf2*, were cloned from a mosquitocidal 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 mosquitocidal 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.

Poster / Bacteria. B-6.

Utilisation of the Rhs core region of *tc-sepC* orthologes as a degenerate system for the rapid amplification of putative insecticidal genes

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Degenerate PCR is an accepted method for obtaining the DNA sequence of a similar gene from a different species or genera. We have successfully used this procedure as a rapid method to obtain the partial DNA sequence of candidate insecticidal genes belonging to the toxin complex (Tc) family. A member of the Tc family - *sepC* and its orthologes are members of a larger family defined as Rhs elements identified in *Escherichia coli*. The DNA of Rhs elements is typified by a conserved GC-rich core and an AT-rich core extension region. Based on the DNA of the conserved core region a series of degenerate primers were designed and used to PCR amplify the partial DNA sequence of tc-sepC like orthologes from known insecticidal *tc-sepC* type containing bacteria and novel insecticidal bacteria of unknown DNA content. Results showed consistent amplification of a ~910-bp DNA segment of the *tc-sepC* gene from various bacterial genomes. This technique not only defines whether a putative insecticidal bacterium contains *tc-sepC* like genes, but also allows determination of the partial *tc-sepC* DNA sequence facilitating the isolation of neighbouring genes that may encode the full compliment of *tc*-like genes.

Poster / Bacteria. B-7.

FlhA flagella basal body protein, influences transcription of PlcR regulated genes, protein production and virulence of *Bacillus thuringiensis*

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Many strains of *Bacillus thuringiensis* (Bt) and *B. cereus* (Bc) are closely related; Bt produce specific insecticidal cry toxins and Bc appears to be an emerging opportunistic human pathogen. It has been shown that Bt and Bc produce many putative virulence factors, which are positively controlled by a pleiotropic transcriptional regulator PlcR. The inactivation of plcR decreases but does not abolish virulence indicating that additional factors may contribute to pathogenesis. A Bt flhA mutant, defective in flagellar apparatus assembly and in motility, was formerly described. We further characterized this mutant in order to understand the relation between motility and virulence. A large picture of secreted proteins, obtained by two-dimensional electrophoresis analysis, reveals that flagellar proteins are not secreted and production of several virulence-associated factors is reduced in the flhA mutant. Moreover, we quantified the effect of FlhA on plcA and hblC gene transcription, significant reduction of these factors were observed in the mutant. Thus, transcription of several PlcR-regulated virulence factors is coordinated with the flagellar apparatus. The flhA mutant also showed a strong decrease in cytotoxicity towards Hela cells and in virulence against greater wax moth, *Galleria mellonella*, larvae following oral or intrahemocelic inoculation. The decrease in virulence may be due to both a lack of flagella and to a lower production of secreted factors

STU Poster / Bacteria. B-8.

Xenorhabdus nematophila secreted proteases and their role in insect pathogenesis

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Xenorhabdus nematophila is a Gram-negative bacterium capable of killing insects. Secreted proteases have been implicated as virulence

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 Zn^{2+} 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 Mg^{2+} transport protein, MgtB from *Salmonella enterica* sv. Typhimurium. Both *prtA*::Tn*5* and *prtX*::Tn*5* 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.

Poster / Bacteria. B-9.

An elastase found in early instar *Lymantria dispar* larvae is involved in the proteolytic activation of *Bacillus thuringiensis* toxins

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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 protoxins 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

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Bacillus thuringiensis ssp. *galleriae* (Bt) is entomopathogen sporeforming bacterias. 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 the12^{th} day and Cat on the 8th day and concentration of GSH / GSSG in 10 times on the 8^{th} 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 12^{th} day, on the 16^{th} 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

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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* 43:14299-305). 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 HevCadLP 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 pIZT 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 killed less than 20% of cells expressing HevCaLP. Therefore, while HevCaLP can be considered a functional receptor for Cry1Ac, the low 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-R_{1a} cadherin enhances activity of Bt Cry1A toxins

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Cadherin-like proteins located in the midgut epithelium of lepidopteran larvae function as receptors for *Bacillus thuringiensis* Cry1 toxins. The cadherin protein Bt-R_{1a} 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 BtR_1 for Cry1Ab binding and cytotoxicity (Hua et al. 2004. *J. Biol. Chem.* 279: 28051-28056). Binding to Bt-R_1 may cause conformational changes in Cry1Ab that lead to oligomerization, binding to aminopeptidase and finally membrane insertion (Bravo et al., 2004. *Biochim. Biophys. Acta* 1667:38-46). We report the discovery that 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 Cry1A toxicity to *M. sexta*, *Heliothis virenscens* 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 Cry1A toxicity enhancement by CR12-MPED will be discussed.

Poster / Bacteria. B-13.

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

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Previously, critical surface-exposed loop residues (P_{389} in β_6-β_7 loop, E_{417} in β_8-β_9 loop, Y_{455} and N_{456} in β_{10}-β_{11}) 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 residues on Cry4Ba toxicity. Double mutants, P389A/E417A (β_6-β_7/β_8-β_9 loops), E417A/Y455A and E417A/N456A (β_8-β_9/β_{10}-β_{11} 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 (P_{389}, E_{417}, Y_{455} and N_{456}) 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

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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-pS136NSSRNP (T6) for the 130-kDa Cry4Ba mutant protoxin containing an additional proteolytic cleavage site in the loop between α3 and α4 were used for producing α4-α5 helical hairpins. The 130-kDa protein inclusions 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 C_{18} column. N-terminal sequencing indicated a correct cleavage site (NPSYRT). A circular dichroism spectrum of these hairpins showed helical structure dissolved in methanol. Membrane permeation studies via calcein release assays revealed that the α4-α5 helical hairpin exhibited high perturbing activity against LUVs (50-70% release) whereas the 65-kDa activated Cry4Ba toxin or mutant toxin (T6) and 47-kDa elution fraction showed relatively low. These results suggested that α4-α5 helical hairpin of the Cry4Ba toxin is involved in pore formation in phospholipid membrane vesicles. ATR-FTIR spectroscopy measurement revealed that the α4-α5 helical hairpins are mainly buried into phosopholipid membranes and show a predominant α-helical structure. Taken together, the data indicate that the α4-α5 helical hairpins play a role in membrane penetration and support the pore-forming "umbrella" model.

STU Poster / Bacteria. B-15.

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

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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 NaH_2PO_4, 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 30-150 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.

Poster / Bacteria. B-16.

Interaction of the Bt toxin Cyt1A with lipid monolayer

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Previously we put forward a hypothesis that, interacting with phospholipids, Cyt1A may act as a surfactant (detergent) rather than a pore former. Here we directly examined detergent-like properties of the toxin by surface tensiometry. First, we determined that Cyt1A decreases surface tension of water from 72.5 mN/m to 45 mN/m. For comparison, a synthetic detergent sodium dodecyl sulfate lowers it to 32 mN/m. Second, we studied Cyt1A-induced surface pressure changes in a lipid monolayer at the air/water interface. Pore-forming toxins are known to insert into the monolayer and increase its surface pressure. We observed that α-hemolysin increased the surface pressure of a 1,2-diacyl-*sn*-glycero-3-phosphocholine (PC) monolayer by 190%. In contrast, the same concentration of Cyt1A increased the monolayer surface pressure by only 21%. The negative control, cytochrome c, a water-soluble protein that does not insert in the lipid monolayer did not change the latters surface pressure. We conclude that Cyt1A exhibits a detergent-like property, namely, it decreases the surface tension of water. It inserts into the lipid monolayer to a significantly smaller extent than α-hemolysin, a known pore-forming toxin. These results are in accord with our previous data, which argued against Cyt1A acting as a pore former.

STU Poster / Bacteria. B-17.

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

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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 bactericidal 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 Cyt1Ca by hydrophobic and non-polar ones; (b) Fusion of this domain to the hydrophobic leader peptide of Mtx1 toxin from *Bacillus sphaericus*. Expressing the generated variants of *cyt1Ca* in *E. coli* exhibited high bactericidal activities which were comparable to that 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*

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Bacillus thuringiensis israelensis is a Gram-positive spore forming bacterium that forms an insecticidal protein toxin crystal that is

composed of several toxin polypeptides which are divided into two families: Cry and Cyt. The Cyt toxins include a main polypeptide, Cyt1Aa and two minor polypeptides Cyt2Ba and Cyt1Ca. Cyt2Ba exists in *Bti* 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 *Bt*IPS78/11. The gene was highly expressed as inclusion bodies which made it easy to obtain pure Cyt2Ba protein. All known toxins of Cyt 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 leaded to cleavage of Cyt2Ba sequence between the 34 and 35 amino acid residues from N-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 camelysin.

Poster / Bacteria. B-19.

Individual characterization of the three *cyt1A* promoters of *Bacillus thuringiensis* subsp. *israelensis*

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During sporulation, *Bacillus thuringiensis* subsp. *israelensis* (Bti) produces a parasporal body that contains four major mosquitocidal proteins, Cyt1A, Cry11A, Cry4A and Cry4B. Among the four proteins, Cyt1A forms the largest crystal, consisting of more than half the parasporal body mass. The principal factors that contribute to this high level of Cyt1A synthesis, are (1) three strong promoters, (2) a strong 3' stem-loop structure and (3) this protein's high stability. However, the characteristics of each *cyt1A* promoter at transcriptional and translational levels have not been evaluated and compared, and virtually no data are available on the activity of the third promoter. Thus, we constructed three recombinant Bti strains with the *cyt1A* open reading frame (orf) under the control of each promoter and a control Bti strain with the *cyt1A* orf under control of all 3 promoters. Expression of these four recombinants was then examined by RNA dot blot analyses, and Cyt1A synthesis was quantified by SDS-PAGE analysis and correlated with spore counts and synthesis per unit medium. Overall, the transcription level of the BtIII promoter was the lowest of the three promoters. SDS-PAGE and spore count analyses also showed that the BtIII promoter strain produced the lowest amount of Cyt1A per spore. The levels and patterns of *cyt1A* expression of the BtI and BtIII promoter strains were significantly different from each other, with BtII producing much more per cell than BtI or BtIII. However, the quantity of Cyt1A produced per unit medium was equivalent statistically among the three test strains and the control. These results indicate that all three promoters are strong promoters, but that BtIII is the weakest of the three, and therefore likely contributes the lowest amount of Cyt1A to the Bti parasporal body.

Poster / Bacteria. B-20.

Analysis of the plasmid replication origin ori165 from *Bacillus thuringiensis* subsp. *tenebrionis*

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A 13kb replication region named ori165 of the large plasmid pBMB175 (90 kb) from *Bacillus thuringiensis* subsp. *tenebrionis* YBT-1765 had been cloned and sequenced, which was the first replication origin isolated from subsp. *tenebrionis*. Deletion analysis indicated that the minireplicon (4.4kb) harbored three open reading frames (ORFs). The ORF1 (518aa) and the putative origin of replication (*ori*) located downstream of ORF1 which were essential for replication of ori165 displayed homology to replication protein and *ori* of pAβ1 family plasmids respectively. By stability test, two overlapping ORFs (ORF6 and ORF10) located upstream of ORF1 were necessary for the stable replication of plasmid. The minireplicon of ori165 could stably replicate for about 40 generation under lacking antibiotic selection conditions in BMB171 and 4Q7, plasmid-cured derivative of *Bacillus thuringiensis* subsp. *kurstaki* and *israelensis*, respectively. The similar replication region was also located in pAW63, pBT9727 (*Bacillus thuringiensis*) and pXO2 (*Bacillus anthracis*). However, three ORFs (ORF2, ORF3 and ORF4) encoded putative transposase gene in ori165 fragment were shown to flank the essential replication region (containing ORF1 and *ori*). By deletion analysis, they were not related to replication. The all evidences suggested that the possibility of the replicons transfer in *B.c* group.

Poster / Bacteria. B-21.

Novel *Bacillus thuringiensis* strains isolated from soil samples in China.

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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.

Poster / Bacteria. B-22.

Diversity of *Bacillus thuringiensis* strains in the maize and bean phylloplane and from their respective soils in Colombia

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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.

Poster / Bacteria. B-23.

Characterization of selected *Bacillus thuringiensis* strains

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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, Pyrausta 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 spore or even larger), harbored *cry1* and were toxic to only 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, yunnanensis* reacted with *cry1* and *cry1, cry2* general primers, correspondingly. Nevertheless they were not toxic to tested insects. *Bt* ssp. *sotto, israelensis, dacota, indiana, kumamotoensis, tochigiensis, mexicanensis, monterrey* appeared small bipyramidal crystals, harbored *cry 7,8* and were nontoxic. Strains of *Bt* ssp. *israelansis* and ssp. *morrisoni* (PG-14) had large irregularly shaped crystals, harbored *cry4, cry11* and were toxic to Diptera. *Bt* ssp. *darmstadiensis* 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.

Poster / Bacteria. B-24.

Effects of *Bacillus thuringiensis* var. *kurstaki* toxins on *Scheloribates praeincisus* (Berlese, 1910) (Acari: Oribatida: Scheloribatidae)

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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.

Poster / Bacteria. B-25.

Effect of *Bacillus thuringiensis* strains on *Spodoptera cosmioides*

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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 cm^2) 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±2ºC, 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.

Poster / Bacteria. B-26.

Study on preparation of *Bacillus thuringiensis* controlling both Lepidoptera and Coleoptera pest

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Recombined plasmid pBMB305-06R was constructed by *cry3A* gene coding Coleoptera-specific protein, and then transformed into a wild *B.t.* strain YBT803-1 by electroporation. A transformant BMBY-001 was obtained. The stabilizer, antiseptic and emulsifier for suspending agent was systematically researched, also the stuffing and accessory ingredient for wettability pulvis was studied and found the best stuffing (D-5) and accessory ingredient (S-1). A new bioassay method was set up for genetic engineering biotic insecticide, and results showed that BMBY-001 was not only highly toxic to *Phyllodecta vulgatissima* larva (LC_{50} 0.413μL/mL) but also toxic to *Plutella xylostella* (LC_{50}3.319μL/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.

Poster / Bacteria. B-27.

Molecular dynamics simulation of *Bacillus thuringiensis* Cry4a mosquito-larvicidal protein in explicit water

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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.

Poster / Bacteria. B-28.

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

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The subterranean burrower bug, *Cyrtomenus 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. *C. bergi* was collected in different field sites in Columbia for the establishment of a laboratory culture. Several specimens died in the laboratory and were sent for diagnosis to the Institute for Biological Control of the Federal Biological Research Centre of Agriculture and Forestry in 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*.

Poster / Bacteria. B-29.

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

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Phytoseiid 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 phytoseiid mites is scarce. In this study, we developed a method to assess adverse effects of Bt toxins on phytoseiid 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.

Poster / Bacteria. B-30.

Impact of *Bacillus thuringiensis* Cry toxins on the predator *Chrysoperla carnea* (Neuroptera: Chrysopidae): *In vivo* binding, histopathological and prey-mediated effects

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Chrysoperla carnea is an important natural enemy of a wide range of crop pests, that is commonly chosen as a control to determine pesticidal side-effects and the ecological impact of *Bacillus thuringiensis* (Bt) crops. Specific binding of ingested Cry1Ac and cell damage was found in epithelial midgut cells of a susceptible insect, *Helicoverpa armigera*. However neither any histopathological effect of Cry1Ac nor inmunodetection of this protein in *C. carnea* was found. The prey-mediated effects of Cry1Ac, Cry1Ab and Cry2Ab on *C. carnea* were studied by feeding them with treated *H. armigera* and *Ephestia kuehniella* eggs. Pupation percentage, proportion of emerged adults and hatching percentage of eggs was no significantly different between treatments. Our results suggest that *H. armigera* fed on Bt crops have not a detrimental effect on *C. carnea* in the field, where the diet is supplemented with other prays like Lepidoptera larvae and eggs, aphids and two spotted mites. We have shown that in the hypothetical case of that Cry1Ac toxin were ingested by *C. carnea*, no binding of this toxin to the midgut of the predator should occur.

Poster / Bacteria. B-31.

Treatment of an *Aedes aegypti* colony during 33 generations with the Cry11Aa toxin of *Bacillus thuringiensis* serovar. *israelensis* results in moderate resistance development

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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 (LC_{50}) of 26.3 ng/ml and at the generation 33 the LC_{50} was 84.6 ng/ml The highest rate of resistance (RR) found was 3.05, detected when the LC_{50} between treated and untreated colonies were compared in generation 33. Kinetic mortality experiments performed with 500 times the Cry11Aa LC_{50} 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*.

Poster / Bacteria. B-32.

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

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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 unselected (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 aliquots 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.

STU Poster / Bacteria. B-33.

A common, but complex, mode of resistance of *Plutella xylostella* to *Bacillus thuringiensis* toxins Cry1Ab and Cry1Ac

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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 Cry1Ab, Cry1Ca and Cry1Da with incomplete mode of inheritance of resistance to Cry1Ac. The Cry1Ab-SEL population showed a marked cross-resistance to Cry1Ac (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.

Poster / Bacteria. B-34.

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

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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 Cry1Ac until 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*.

Poster / Bacteria. B-35.

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

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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.

Poster / Bacteria. B-36.

Reduction in levels of the *Heliothis virescens* alkaline phosphatase (HvALP) as a marker for resistance to Cry1Ac

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We previously reported detection of alkaline phosphatase (HvALP) that bound Cry1Ac toxin in brush border membranes from *Heliothis virescens*. Both alkaline phosphatase-specific activity and HvALP protein levels were reduced in Cry1Ac-resistant larvae of the *H. virescens* YHD2 strain, but not in susceptible offspring from backcrosses, suggesting a direct correlation between HvALP levels and Cry1Ac resistance (Jurat-Fuentes and Adang 2004, *Eur. J. Biochem.*, 271:3127-35). In *H. virescens* brush border membrane proteins separated by 2-dimensional (2D) gel electrophoresis, we identified HvALP as a chain of protein spots recognized by antiserum against membrane-bound ALP. In qualitative comparisons of HvALP in susceptible (YDK) and resistant (YHD2, CXC, and KCBhyb) *H. virescens* strains using 2D immunoblots, reduced amounts of HvALP were detected in all resistant strains. Alkaline phosphatase activity was significantly reduced in all resistant strains when compared to susceptible samples, further evidence for a direct correlation between reduced HvALP levels and Cry1Ac resistance. Furthermore, HvALP and Cry1Ac, together with cadherin, are co-localized in lipid rafts in the brush border membranes of *H. virescens*. These results support the potential use of HvALP alterations as a marker for Cry1Ac resistance in *H. virescens*.

Poster / Bacteria. B-37.

Could insect gut esterases be a threat to Bt crops?

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A number of insects have been shown to develop resistance to specific *Bacillus thuringiensis* (Bt) Cry toxins under laboratory selection and only one species, *Plutella xylostella*, has developed widespread resistance to Bt products under the intensive open field selection commonly found in crucifer crops. There is evidence that additional mechanisms of resistance may be present in some insect populations. Recent work on *Helicoverpa armigera* has provided evidence of metabolic resistance mechanism due to over-production of esterases, which sequester Bt toxins before they reach its target. We therefore explored the possibility of interaction between Cry1Ac and esterase by selecting *P. xylostella* population (SERD4) with Cry1Ac and deltamethrin. Deltamethrin selection for seven generations increased resistance relative to the unselected sub-population >10,000-fold, with significant cross-resistance to Cry1Ac

(>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 Cry1Ac.

STU Poster / Bacteria. B-38.

Analysis of midgut proteinases from *Bacillus thuringiensis* susceptible and resistant *Heliothis virescens* (Lepidoptera: Noctuidae)

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Heliothis virescens is a major lepidopteran pest of cotton in the United States and the target insect of *Bacillus thuringeinsis* (Bt) transgenic cotton. We conducted an analysis of gut proteases 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

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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 level of potency. We also demonstrate that a single set of potentiators can potentiate several Class A proteins.

Poster / Bacteria. B-40.

Novel toxin complex constructions

Timothy Hey, Charles Cai, Aaron Woosley, Stephanie Burton, Joel Sheets, Brian Waldman, Haley Moon, Tom Meade and Don Merlo

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The Toxin Complex proteins from *Photorhabdus* 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.

Poster / Bacteria. B-41.

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

Larry Gringorten[1], Young Choi[2], Lyne Morel[2], Luke Masson[2] and Carlos Miguez[2]

[1]Great Lakes Forestry Centre, Canadian Forest Service, 1219 Queen St. E., Sault Ste. Marie, Ontario, P6A 2E5, Canada, [2]Biotechnology 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 bipyramidal, 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.

WEDNESDAY - 10 August

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

Genomics of entomopathogenic nematodes and symbiotic bacteria

Symposium. 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. 90

***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. 91

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

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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 insect 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. 92

***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. 93

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 GSP11 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 rnc(-) mutant of *P. luminescens* TT01 HU1956 and NS107 strains to increase the frequency of RNAi by using rnc(-) mutants. Primers were designed for the Tc1 transposase of *C. elegans*.

Symposium. Wednesday, 9:40. 94

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]

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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) symbiont 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* (HSP60 chaperonin), *daf-21* (HSP90), *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 symbiont specific colonization of the nematode gut and growth and development as well as insect parasitism.

CONTRIBUTED PAPERS. Wednesday, 8:00-9:45

FUNGI 2

Contributed paper. 95

Withdrawn

Contributed paper. Wednesday, 8:00. 96

A molecular diagnostic method for selected *Ascosphaera* species using PCR amplification of internal transcribed spacer regions of rDNA

K. Daniel Murray[1], Katherine A. Aronstein[2], and Walker A. Jones[3]

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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 other *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.

STU Contributed paper. Wednesday, 8:15. 97

Multiple genetic lineages coexist sympatrically within a local population of *Beauveria bassiana* s. l.

Nicolai V. Meyling[1], Stephen A. Rehner[2], Mette Lübeck[3], Ellen P. Buckley[2] and Jörgen Eilenberg[1]

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Isolates of *Beauveria* spp. from arable field soil of one organic agroecosystem in Denmark as well as isolates from soil, insects and phylloplanes of selected plants from the surrounding hedgerow were characterised by PCR. Characterisations were made by sequencing specific genomic regions (EF1-alpha; B-locus) recently found to be variable within *Beauveria* spp. and *B. bassiana*, respectively. Additionally, multiple microsatellite loci were amplified for *B. bassiana* isolates. In the hedgerow, *B. bassiana* was found in soil, in insects and on phylloplanes. No apparent associations between isolation site and genetic grouping were found. Totally, the hedgerow contained 4 clonal lineages and one possibly recombining linage of *B. bassiana*, but also *B. brongniartii* and members of a recently identified *B. bassiana*-like clade. All three "species" clades were harboured in the hedgerow soil. In contrast, all isolates from arable field soil belonged to a single linage of *B. bassiana* previously described to be common in European agricultural systems. The study documents an extraordinary local diversity of *Beauveria* spp. in the hedgerow habitat contrasted by lack of diversity in neighbouring agricultural soil. Specific strains are possibly adapted to certain conditions in arable soils and hedgerow habitats are thus important reservoirs for indigenous *Beauveria* diversity.

Contributed paper. Wednesday, 8:30. 98

Characterization of a *Beauveria bassiana* isolate from feral black-legged ticks, *Ixodes scapularis* (Say)

Lina B. Flor, Timothy J. Kurtti and Ulrike G. Munderloh

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We isolated a fungus from a black-legged tick, *Ixodes scapularis*, collected from a white tailed deer. The isolate, Bb-Is2, was identified as *Beauveria bassiana* based on microscopy and molecular analyses. The primer set ITS4 and ITS5, targeting regions flanking ITS1 and ITS2 of *B. bassiana* nuclear rDNA, amplified a 600 bp PCR product. The ITS sequences were closely related to *B. bassiana* isolates from Chrysopidae, Aphididae and Aranea collected in different geographic areas. The primers NS5 and NS6, specific for the nuclear small subunit rRNA gene, yielded a 300 bp PCR product and its sequence demonstrated that Bb-Is2 lacks the group I intron. The optimum temperature for germination and growth was 25ºC with a mean diametric growth rate of 3.4 mm/day and a 97% germination rate. There was no germination at 4, 34 or 37ºC. Larvae showed maximum mortality 28 days post infection. Younger unfed larvae (36 days old) displayed a higher mortality (75%) than older (156 days old) unfed larvae (20%). Engorged larvae treated 2-3 days post repletion were more susceptible (80% mortality) than unfed ticks or those about to molt to nymphs. Further investigations are ongoing to determine the susceptibility of fed and unfed nymphs.

STU Contributed paper. Wednesday, 8:45. 99

The role of oxalic acid in the pathogenicity of *Beauveria bassiana* towards Ixodidae tick species

Brett Kirkland and Nemat O. Keyhani

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

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.

STU Contributed paper. Wednesday, 9:00. 100

Iron acquisition in the entomopathogenic fungus *Beauveria bassiana*

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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 ferricrocin. 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.

Contributed paper. Wednesday, 9:15. 101

Comparison of different methods for isolation of entomopathogenic fungi from soil

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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 for *B. bassiana* and *M. anisopliae*. The best results were obtained from SDM for both species of fungi. 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. Wheras from the forest soil: SDM - 82% for *B. bassiana*, 100% for *M. anisopliae*; 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.

Contributed paper. Wednesday, 9:30. 102

Virulence and sporulation of *Metarhizium anisopliae* in the presence of *Trichoderma conidia* on agar substrates and in soil bioassays on larvae of the black vine weevil

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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 coinoculated with *T. atroviride* at 20-22ºC. 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 20ºC, despite germination and growth optima at 25-30ºC. 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 22ºC, 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.

CONTRIBUTED PAPERS. Wednesday, 8:00-10:00

IMMUNITY

Contributed paper. Wednesday, 8:00. 103

***Spodoptera littoralis* response to infection with AcMNPV**

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We studied the infection of *S. littoralis* larvae by the *Autographa californica* 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 proleg *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* larvae immunosuppressed by the endoparasitic wasp *Chelonus inanitus* became more susceptible to AcMNPV 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*.

STU Contributed paper. Wednesday, 8:15. 104

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

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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.

Contributed paper. Wednesday, 8:30. 105

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

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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 parasitoids, 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*, supresses hemolymph melanization and PPO activity. This inhibition of PPO activity in *C. fumiferana* can be reproduced by injecting in the hemocoel 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 CfPPO1 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. 106

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

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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 the 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 immunosuppression of *M. sexta*. Our research will clarify the importance of CcV1 protein expression in mediating cell death of insect immune cells, which weakens the immune system of *M. sexta*.

Contributed paper. Wednesday, 9:00. 107

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

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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 boulardi*, a natural parasite of *Drosophila*. The infestation-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. 108

Investigating immune functions in mosquito cell lines

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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. 109

Transcriptome studies on the penaeid shrimp biodefense-related genes

Takashi Aoki[1], Ikuo Hirono[1], Motoshige Yasuike[1], Koolvara Sangrungrunang[2], Ryuji Ueno[3], Lila Ruangpan[4], Yukinori Takahashi[5], Ratree Wongpanya[6], and Anchalee Tassanakajon[6]

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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, *Marsupenaeus japonicus* and black tiger shrimp, *Penaeus monodon* to discover immune-related genes. Based on these ESTs, we constructed a cDNA microarray (spotted 1,026 distinct clones) of kuruma shrimp and black tiger shrimp. In this study we analysed the gene expression profile of the immune response of black tiger shrimp orally fed with antibiotics oxytetracycline (OTC) or oxolinic acid (OA), and artificially infected with white spot disease virus (WSV) 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 OTC or OA and after aritificial infections of WSV or *V. harveyi*. Most of these genes (known and unknown functions) were down-regulated by the administration of antibiotics. In the case of pathogen infection, several gene expressions were changed, i. e.

expression patterns in the shrimp hemocytes were different beween bacterial and viral infection.

Contributed paper. Wednesday, 9:45. 110

Characterization and expression analysis of biodefense-related genes from kuruma shrimp, *Marsupenaeus japonicus*

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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_

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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 chips, 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 Herrero[1, 2], Marleen Ansems[2], Monique M. van Oers[2], Just M. Vlak[2], Petra L. Bakker[1], William J. Moar[3], and Ruud A. de Maagd[1]

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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.

Symposium. Wednesday, 11:30. 113

The infection and cell specific replication of the most successful viral insecticide, *Anticasia gemmatalis nucleopolyhedrovirus* (AgMNPV), in its host

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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 intrahaemocoelic innoculation. vApAg also induces apoptosis in a cell culture derived from *A. gemmatalis* (UFL-AG-286), abrogating protein synthesis at late times post-infection and reducing viral progeny production. Apoptotic bodies and entire cells were phagocytosed by plasmatocytes 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.

Symposium. Wednesday, 12:00. 114

Immune depression triggered in insects by the bacteria *Xenorhabdus nematophila* and *Photorhabdus luminescens*

Michel Brehélin, Robert Zumbihl, Karine Brugirard, Fabienne Vigneux, Carlos Ribeiro and Alain Givaudan

Ecologie Microbienne des Insectes et Interactions Hôtes-Pathogénes, INRA-UMII Pl. 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 pathogen 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 (Brehélin 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 immunocytes (haemocytes) as main targets. There is a high redundancy in the kinds of secreted immunodepressive toxins and in their modes of action.

CONTRIBUTED PAPERS. Wednesday, 10:30-12:30

NEMATODES

Contributed paper. Wednesday, 10:30. 115

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

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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. Siegel[1], Lawrence A. Lacey[2], Patricia Noble[1], James Bettiga[3], Bradley Higbee[4], and Robert Fritts, Jr.[5]

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Previously, we demonstrated that in small plot studies, infective juveniles (IJs) applied at a density of one billion per hectare and application rates of 1,800-3,740 liters per hectare followed by wetting the nuts at that same rate produced 60-90% mortality of navel orangeworm larvae inside infested pistachios. In this study we were interested in assessing nematode efficacy at an application rate orchard managers would use. IJs were applied at a concentration of 1.235 billion per hectare using two different methods of application, chemigation and herbicide sprayer. Chemigation was assessed in one 32.4 hectare pistachio block and herbicide sprayer application (1,892 liter capacity and an application rate of 1,870 liters per acre) was assessed in two 32.4 hectare pistachio blocks. Nematode efficacy was evaluated in all trials by comparing adult emergence from nuts collected prior to application and one week after application. Infested sentinel nuts were also used in the herbicide applicator study. In one block, mortality in nematode treated sentinels was 53.6% (59/110) compared to 19.0% control mortality (8/42) and in the second block mortality in nematode treated sentinels was 66.5% (105/158) compared to 28.9% control mortality (37/128). Analysis of adult emergence is ongoing.

STU Contributed paper. Wednesday, 11:00. 117

Laboratory characterization of *Steinernema carpocapsae* , *S. feltiae* , and *Heterorhabditis bacteriophora* for a multi-species biological control approach targeting the alfalfa snout beetle, *Otiorhynchus ligustici* (L.)

Gabor Neumann and Elson Shields

Cornell University, Ithaca, USA

S. carpocapsae NY001, *S. feltiae* Valko, and *H. bacteriophora* Oswego were evaluated in the laboratory with a multi-species focus to control the alfalfa snout beetle, *Otiorhynchus ligustici* . Multi-well plate penetration assays were used to assess the ability of the nematodes to enter different life stages of the host. Single-piece sand column assays were used to assess the host finding ability of the nematodes. Multi-piece sand column assays were used to investigate the dispersal of the nematodes and the competition for hosts among the nematode species at different depths. *S. carpocapsae* NY001 showed the highest penetration in all tested life stages of the alfalfa snout beetle (adult, first, and fourth instar larvae) while *H. bacteriophora* Oswego showed the lowest penetration rates in all life stages. *S. feltiae* Valko showed intermediate penetration rates but could not be separated statistically from *S. carpocapsae* NY001 in the adult beetle, from *H. bacteriophora* Oswego in the first instar larva, and from either of the nematodes in the fourth instar larva. Penetration rates in single-piece sand columns significantly differed in all three nematodes *H. bacteriophora* Oswego having the highest penetration rate and *S. carpocapsae* NY001 the lowest penetration rate. *S. feltiae* Valko again showed intermediate penetration rate. In the multi-piece sand column assays, all three nematodes were capable of infecting hosts down to the 32.5 cm depth when applied alone. In nematode combinations, competition occurred but the nematodes did 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 6.5 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.

Contributed paper. Wednesday, 11:15. 118

Improvement of *Steinernema carpocapsae* for control of the pecan weevil, *Curculio caryae* through hybridization and bacterial transfer

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Steinernema carpocapsae has shown promise for control of the pecan weevil, *Curculio caryae*, a key pest of pecan. Previously, the Italian strain of *S. carpocapsae* was found to be among the most virulent to *C. caryae*, but had poor heat and desiccation tolerance. In contrast, the DD-136 strain exhibited high levels of heat and desiccation tolerance but low virulence. Our objective was to determine the feasibility of developing improved *S. carpocapsae* strains by transferring the nematode's bacterial symbiont from the Italian strain to the DD-136 strain, and through hybridization between the two wild type nematodes. Three modified strains were created: one through bacterial transfer alone and two hybrids through controlled crosses. We hypothesized that the improvement approaches would result in strains possessing high levels of environmental tolerance similar to DD-136 and virulence similar to the Italian strain. The hypothesis was supported in two out of three modified strains. Heat and desiccation tolerance in the three modified strains was more than 2.5 fold greater than the Italian strain and not different from the DD-136 strain, except one hybrid had lower heat tolerance than DD-136. Virulence to adult weevils in the modified strains was similar to the Italian strain and greater than DD-136. Overall, the results indicate

that bacterial transfer and hybridization could be a valuable tool in improving biocontrol efficacy of steinernematids.

STU Contributed paper. Wednesday, 11:30. 119

Evaluating efficacy of application of entomopathogenic nematodes in drip-line irrigation systems

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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.

Contributed paper. Wednesday, 11:45. 120

Soil biology of entomopathogenic nematode, *Steinernema abbasi* and the impacts of host plants on its pathogenicity

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The impacts of abiotic factors on the survivorship of infective juveniles (IJs) of *S. abbasi*, was studied. In addition, the vertical and horizontal movements of *S. abbasi* in sandy loam soil were examined. Finally, the pathogenicity of *S. abbasi* towards the different host plants-fed *Spodoptera litura* larvae was investigated. Forty-eight and 72 hours after *S. abbasi* applied in sandy loam soil and placed at 15 - 40ºC at 5 degree interval, the survival rates were decreased gradually. The survival rate of IJs when placed at 15ºC was 70%. And no live nematode was found when infective juveniles of *S. abbasi* were placed at 40ºC. As infective juveniles of *S. abbasi* placed in soil at pH of 4, 7, and 10, the survival rates was ca. 12%. The survival of *S. abbasi* was zero sixty minutes later for rapidly dehydration (y=0.9167X2-25.405X+134.57). On the contrast, ninety minutes after *S. abbasi* slowly dehydrated, the survival rate was ca. 60% (y=-2.7083X2-13.256X+83.161). The impact of canopy on the survival of S. abbasi was also investigated in these studies. In the field trial, IJs were applied on the soil surface of sweet potato field at 8:00 and 16:00, higher survival rate of *S. abbasi* was obtained from sheltered when compared with exposed field. As compared the coverage effect of various host plants, the survival rates of *S. abbasi* were in the order of sweep potato, tomato and cabbage. For the straw and PE materials, lower survival rates were obtained from straw and PE coverage as compared with the data of control. Most of the IJs have stayed at the soil surface in both vertical and horizontal movement and very few IJs have moved downward or laterally as time has proceeded. However, the movement of IJs was never deeper than 15 cm. The number of IJs entered into the larvae of *Spodoptera litura* was increased as the time proceeded. The numbers of IJs emerged from host larvae (= yield) is positive related with the body weight of infected host larvae (Y = 187684 X2 - 83922X + 36933). The mortality of *S. litura* larvae was zero at the first 6~12 hours after inoculation no matter the food they have fed, and reached a mortality of 98% 72 hours after. The pathogenicity of *S. abbasi* to *S. litura* larvae fed with various host plants was in the order of taro, lettuce, tomato and cabbage.

STU Contributed paper. Wednesday, 12:00. 121

Infection preferences of an entomopathogenic nematode, *Steinernema riobrave*

Jayne M. Christen[1], James F. Campbell[2], and Sonny B. Ramaswamy[1]

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Entomopathogenic nematodes are lethal endoparasites of insects. Infective juveniles (IJs) 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 over 24 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.

Contributed paper. Wednesday, 12:15. 122

Movement, colonization, and persistence of the entomopathogenic nematode *Heterorhabditis marelatus* in a California coastal grassland

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In a California coastal grassland, the entomopathogenic nematode *Heterorhabditis marelatus* indirectly protects the bush lupine *Lupinus arboreus* from herbivory by preying upon root-boring larvae of the ghost moth *Hepialus californicus*. This interaction is affected by climate; *H. marelatus* is prone to desiccation and must survive dry soil and low prey availability during the dry summer season. Observational and experimental studies have shown that nematode survival in the moist soil under lupines is higher than in the surrounding grasslands, but long-term surveys show high rates of local extinction and interannual variability in population sizes even in favorable microhabitats. In the winter of 2004, we set up a spatial array of almost 900 lupine saplings within 5-gallon, bottomless buckets buried in the soil. Biomass of lupines and incidence of ghost moths were monitored regularly, and, in the spring of 2005, EPN incidences within and outside these rhizospheres were assayed with a new field technique using *Galleria mellonella* baits in 15 mL centrifuge tubes. Lab trials and comparisons with simultaneous soil samples showed that this method is a highly sensitive tool for detection of EPN populations in the field. While ghost moth incidence was very low on experimental lupines (<5%), over 70% of rhizospheres and 25% of grassland samples tested positive for *H. marelatus*. Although *H. marelatus* is found rarely in grasslands during dry summers, populations may expand from lupine refuges during the wet winter season to colonize unoccupied rhizospheres. Experiments demonstrate that infective juveniles can move on their own through soil more than a meter within weeks, and that non-host isopods, millipedes, and snails can transport IJs phoretically over longer distances. Continuing experiments will test the hypotheses that cues from host insects and lupine roots induce directional dispersal and colonization.

CONTRIBUTED PAPERS. Wednesday, 10:30-12:30

MICROBIAL CONTROL 1

Contributed paper. Wednesday, 10:30. 123

Sphaerus® SC, a Brazilian bioinsecticide to control the vector of malaria and filariases

[1]Rose Monnerat and [2]Carlos Marcelo Soares

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Sphaerus® SC is a bionsecticide developed through a cooperation between Embrapa and Bthek 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 mosquitoes and black-flies

Rose Monnerat [1] and Carlos Marcelo Soares[2]

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Bt-horus® SC is a bioinsecticide developed through a cooperation between Embrapa and Bthek 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 Health Ministry under the number 3.2813.0002.002-9. It has been used to control black flies in many farms including Embrapa's experimental farm, in hotels located in rural and tourist areas and in the official residence of the President of the Republic. It was also tested in water containers with a water replacement regime to control *Aedes aegypti* larvae resulting in 1 month 100% control of *Aedes* population.

Contributed paper. Wednesday, 11:00. 125

Controlled delivery of single and joint-action biolarvicide formulations for control of mosquito larvae

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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. Corn 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

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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 results 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. Shternshis[1], Maxim A. Vaskin[1], Vladimir V. Gouli[2]

[1]Novosibirsk State Agrarian University, Russia, and [2]University of Vermont, USA

Two formulations registered in Russia were tested to the control of Siberian populations of blackcurrant insect pests: gooseberry fruit-worm, *Zophodia convolutella*, gooseberry aphid, *Aphis glossulariae* and currant aphid, Capitophorus 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 *Streptomyces avermitilis* biomass were used for evaluation. Laboratory experiments had 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 the significant efficacy of both formulations for *Z. convolutella*, and Phytoverm® for *A. glossulariae*. 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 population restoration for a short period, whereas Lepidocide® treatment appeared no influence on these beetles. Thus, two microbial formulations are efficient for controlling the blackcurrant pests under the tested conditions.

Contributed paper. Wednesday, 11:45. 128

Mortality of gypsy moth (*Lymantria dispar*) induced by *Bacillus thuringiensis* var. *kurstaki* is inversely related to temperature

Kees van Frankenhuyzen

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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 25ºC to ~80 h at 13ºC. The 50% lethal dose 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 22ºC. Rearing temperature was varied from 13 to 25ºC 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 Li[1,2], Jianxin Tan[1], Lanlan Han[2], Kanglai He[1], Gemei Liang[1], Fuping Song[1], Dafang Huang[3], Jie Zhang[1]

[1]State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100094, [2]College of Life Sciences, Northeast Agricultural University, Harbin, 150030, [3]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 134kDa molecular weight. As a new holo-type *cry* gene, this gene was named *cry1Ah1* (Accession number AF281866) by Bt δ-Endotoxin Nomenclature Committee. It could be expressed normally in Bt acrystalliferous mutant HD-73- 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 Cry1Ah1 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 Yu[1,2], Fuping Song[1], Jie Zhang[1], and Jjguo Gao[2]

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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 .161kb .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 best 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, and Gs may mean something to a blob of tRNA 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 insectidal 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 *Heliothis virescens* (tobacco budworm) and *Helicoverpa zea* (cotton bollworm).

STU Contributed paper. Wednesday, 2:15. 136

Identification of vip3A-type genes from *Bacillus thuringiensis* strains and characterization of two novel vip3A-type genes

Jinhuan Liu[1,2], Fuping Song[1], Jie Zhang[1], Jianxin Tan[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]Agricultural 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 *Hin*d III and *Eco*R I 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 BtAL displayed a novel RFLP pattern of 876-bp, 260-bp, 160-bp and 160-bp fragments while 15 isolates including strain Bt41 had another new RFLP pattern of 1146-bp, 155-bp and 155-bp fragments. The full length of *vip3A* gene of BtAL containing 2409-bp was obtained and shared 96% sequence homology with *vip3Af1* (*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 product was toxic to the Lepidoptera larvae. 1456-bp fragment of *vip3A* gene in Bt41 was cloned by PCR method and it shared 83% sequence homology with *vip3Af1* (*isp3a*) gene.

Contributed paper. Wednesday, 2:30. 137

Two novel classes of secreted insecticidal proteins of *Bacillus thuringiensis*

William P. Donovan[1], James T. Engleman[2], Judith C. Donovan[2], William P. Clinton[1], Oliver M. Ilagan[1], Karina C. Krasomil-Osterfeld[1], Thomas M. Malvar[1], John W. Pitkin[1], Matthew R. Walters[1], James A. Baum[1], and James K. Roberts[1]

[1]Monsanto Company, St. Louis, MO 63017, [2]Ecogen Incorporated, Langhorne, 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 distantly related (49% identity) to the toxin portion of the *B. thuringiensis* Cry1Ca protein.

Contributed paper. Wednesday, 2:45. 138

Enterotoxigenic genes: Are they involved in insecticidal activity in *Bacillus thuringiensis*?

George Kyei-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 diarrhial 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-*CytK* 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, *Lymantria dispar* and colorado potato beetle, *Leptinotarsa decemlineata*. We will discuss the possible implications in deleting these virulence traits

STU Contributed paper. Wednesday, 3:00. 139

Cloning and mutation of the cry8C-type gene from *Bacillus thuringiensis* toxic *Anomala corpulenta*

Changlong Shu[1], Fuping Song[1], Shuliang Feng[2], Rongyan Wang[2], Jie Zhang[1]

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A novel *cry8C*-type gene was detected and cloned from a new *Bacillus thuringiensis* strain HBF-1 which had insecticidal activity against the fifteen days *Anomala corpulenta* larvae. This *cry8C*-type gene, designated *cry8Ca2*, consisted of an open reading frame of 3,480 bp and encoded a protein of 1,160 amino acid residues with predicting molecular mass of 130.5 kDa. When the Cry8Ca2 toxin was expressed in a crystal negative *B. thuringiensis* strain HD-73$^-$, spherical crystals were produced and cell extracts from this recombinant strain appear to have insecticidal activity against the fifteen days *Anomala corpulenta* larva (LC50=6.45—10^8CFU ml^{-1}). By Random-mismatch PCR method, 44 mutants of the *cry8Ca2* gene were constructed and expressed in *B. thuringiensis* strain HD-73$^-$. Bioassay results indicated that two of them exhibited higher insecticidal activity against *Anomala corpulenta* larva. The mutant 102 with an amino acid changed in domain II on position 439 (Q to P) caused 5-fold increases in toxicity and mutant 100 had an amino acid changed in amphipathic helices of domain III on the position 642 (E to G) resulted in 4.5-fold increases in toxicity.

STU Contributed paper. Wednesday, 3:15. 140

Introduction of *Culex* toxicity to lepidopteran specific *Bacillus thuringiensis* Cry1Aa by protein engineering

Xinyan Liu, Donald H. Dean

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Deletions and substitutions were designed and constructed in lepidopteran-specific Cry1Aa to mimic the loop regions of mosquitocidal Cry4Ba. Toxicity to mosquito *Culex pipiens* was introduced to Cry1Aa at µg/ml level, while toxicity to its natural target insect *Manduca sexta* was abolished. The successful grafting of the alternate mosquito toxicity onto the original lepidopteran Cry1Aa toxin by exchanging the specificity-determining loop regions demonstrates that combination of sequence alignment and molecular modeling can lead the rational design and engineering of desired toxicity into any toxin of a common scaffold by reshaping the receptor binding region with preferred specificities.

STU Contributed paper. Wednesday, 3:30. 141

The activity of antioxidants in midgut of larvae *Galleria mellonella* infected by *Bacillus thuringiensis*

Ivan M. Dubovskiy , Ekaterina V. Grizanova, Elena A. Boiarisheva, Viktor V. Glupov

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The effect of toxins of bacterium *Bacillus thuringiensis* on epithelial cells of insect midgut is well known. However the resistance mechanisms of insects in particular the role of antioxidants in bacterial disease are practically not investigated. The concentration of malondialdehyde (MDA, lipid peroxidation product), the activity of fermentative antioxidants such as superoxid dismutase (SOD), glutathione-S-transferase (GST), catalase and concentration such nonfermentative antioxidant as tiols in midgut of larvae *Galleria mellonella* were investigated during pathogenesis caused by *B.thuringiensis* ssp. *galleriae* strain 69-6 (BT). We have registered the increase in the oxidized tiols to reduced tiols (RSSR/RSH) ratio and both SOD and GST activities in larvae midgut after infecting with BT. In addition, the bacterial infection lead to significant increase of MDA concentration and mortality of larvae on the first day and to decrease MDA concentration on the second and third day post inoculation. These results have suggested that the increase of lipid peroxidation activity in the cells of larvae midgut under the influence of BT toxins result in formation of toxic metabolites and reactive oxygen species (ROS). Probably the antioxidants activity in the midgut of insects infected BT allows to control the development of bacterial disease due to inhibition of ROS and toxic metabolites.

Contributed paper. Wednesday, 3:45. 142

Assessing the non-target impacts of Foray 48B on soil biota

M. O'Callaghan[1], S.U. Sarathchandra[2], N.L. Bell[2], L.T.Davis[2] and E.M Gerard[1]

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The effects of Foray 48B (*Bacillus thuringiensis kurstaki, Btk*) on soil biota were assessed in pots growing ryegrass/whiteclover. Soil bacterial, fungal and nematode populations were enumerated at 1, 2 and 4 weeks after treatment with three rates of Foray. Bacterial genetic and functional diversity were determined by PCR-amplification of bacterial 16S genes combined with denaturing gradient gel electrophoresis and bacterial physiological profiling respectively. Total fungal and nematode populations in soil treated with field rate (FR) and 100x FR did not differ significantly from untreated soil; total bacterial numbers reflected the application of *Btk*. The soil bacterial functional diversity of bacteria in 1000x FR treatment was significantly different at one week, but after two weeks, functional diversity was similar to other treatments. DNA fingerprinting patterns showed that Foray application had no impact on the diversity of the indigenous soil bacterial community. Using *Bacillus*-specific PCR primers, bands corresponding to *Btk* were detected within the natural soil populations of bacilli only at 100 and 1000x FR. Total nematode abundance increased only at 1000x FR, with bacterial-feeding Rhabditidae nematodes increasing significantly at two weeks. Foray may have provided a food source for these nematodes, limiting the persistence of high densities of *Btk* in the soil.

Wednesday, 2:00-4:00

POSTERS – 2

VIRUSES

Poster / Viruses. V-1.

Heterologous baculovirus pathogenicity in the absence of contemporary coevolution

Gaétan Moreau[1], Christopher J. Lucarotti[1], Edward G. Kettela[1], Kevin N. Barber[2], Stephen E. Holmes[1], Stephen B. Holmes[2], Charles Weaver[1], and Benoit Morin[1]

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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

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.

Poster / Viruses. V-2.

Ecosystem alteration modifies the relative strengths of top-down and bottom-up forces in a herbivore population

Gaétan Moreau[1,2], Eldon S. Eveleigh[1,2], Christopher J. Lucarotti[1,2], and Dan T. Quiring[2]

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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

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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^{10} 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.

Poster / Viruses. V-4.

Relative activity of baculoviruses of the diamondback moth

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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

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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 *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) at titers 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.

Poster / Viruses. V-6.

Fall armyworm *Spodoptera frugiperda* base line of susceptibility to baculovirus SfNPV strain from Paraná , Brasil

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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 supress 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 *Sf*NPV 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 "castorseed" 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 LC95 was of 735,968 between the limits 181,755 and 7'223,208. 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.

Poster / Viruses. V-7.

RAPD-PCR fragments marking resistance and susceptibility of *Lymantria dispar* to nuclear polyhedrosis virus

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For experiments the hundred gypsy moth larvae (3-rd instar) were infected with NPV ("VIRIN-NSH" formulation) in dose of 104 inclusion bodies per larvae. 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 denaturizing during 5 min at 95ºC with following 45 cycles by the following scheme: 95ºC (1 min), 36ºC (1 min), 72ºC (2 min). The reaction was terminated after 10 min at 72ºC. 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 17812017 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 17596555 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 fractions 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

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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 LdNPV 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.

Poster / Viruses. V-9.

In vitro* propagation of NPVs from *Lymantria xylina

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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 LyxyNPV, had been found, these NPVs had been propagated in cell lines. Both NPVs showed 50-80% infection rate (PIB-forming cells/infected cells) to LD cells but LyxyNPV 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 LyxyNPV 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 LyxyNPV 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 LyxyNPV, existed in Taiwan's *L. xylina* populations and LY2 cells might be an available tool for studying the persistent infection of NPV.

Poster / Viruses. V-10.

Genetic stability of *Erinnyis ello* granulovirus applied as a bioinsecticide in Brazil

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The *Erinnyis* Baculovirus was first applied in the middle 80's as a bioinsecticide to the control of cassava pest in Brazil, mainly in Santa Catarina State (SC). In the present work the genetic stability of this virus was monitored using restriction endonuclease analysis. Six seasonal isolates of *Erinnyis ello* granulovirus were obtained from larvae collected in the region of Itajai/Jaguruna (SC) from year 1986 to 2000. Viral DNA analysis showed a total of twenty, twelve, four, eight and four fragments produced respectively by cleavage with Eco RI, Eco RV, Pst I, Bam HI and Hind III enzymes. Submolar bands were detected in all digestions, except for Hind III. A similar flow in the viral population was observed on the Eco RI and Pst I generated profiles. From year 1999 it was evidenced that a 13.6 Kb band turned to be present (Eco RV profile) while a 2.4 Kb band disappeared (Bam HI profile). Moreover, the variation of the intensity of a 8,6 Kb and a 12,7 Kb submolar band, observed by Eco RI and Pst I digestion respectively, confirmed a great change in year 1999. This result indicate that a new genotype (s) became predominant in the viral population since that year.

Poster / Viruses. V-11.

Coral red fluorescence protein as genetic modified baculovirus tracer

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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.

Poster / Viruses. V-12.

Short term starvation reduces intrastadial developmental resistance of gypsy moth (*Lymantria dispar*) to LdNPV

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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 midpoint 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.

Poster / Viruses. V-13.

The GP64 protein of AcMNPV rescues HaSNPV transduction in mammalian cells

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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 (HaSNPV) 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, HaSNPV 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 HaSNPV. Even with lower transduction ratios overall, the range of transduced mammalian cell types with the HaSNPV 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.

Poster / Viruses. V-14.

A cellular *Drosophila melanogaster* protein with similarity to baculovirus envelope fusion proteins

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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 in 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 *f* gene, the function and localization appears to have changed substantially upon adaptation to the baculovirus infection cycle.

Poster / Viruses. V-15.

Analysis of the CfMNPV IAP genes

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Choristoneura fumiferana multiple nucleopolyhedrovirus (CfMNPV) productively infects the the *C. fumiferana* cell-line Cf-203 whereas infection by the type baculovirus *Autographa californica* MNPV induces apoptosis within the first 24 hours. In order to investigate what controls this block in host range, the CfMNPV inhibitor of apoptosis genes (*iap*s) were studied. CfMNPV encodes three genes from the *iap* class of proteins: *iap-1, iap-2* and *iap-3*. All three putative IAP genes have conserved baculovirus early promoters in their upstream region and all putative gene products contain a C-terminal ring finger, while IAP-1 and IAP-3 have one and two copies of baculovirus iap repeats respectively. Phylogenetic analysis revealed the CfMNPV IAPs were most closely related to those from *Orgyia pseudotsugata* MNPV, *Epihyas postvittana* NPV and *Hymantria cunea* NPV. Additionally, CfIAP-3 clustered within a group which included lepidopteran iaps from *Trichoplusia ni, Spodoptera frugiperda* and *Bombyx mori*, suggesting that CfIAP-3 may have been acquired from a lepidopteran host. Interestingly, AcMNPV does not encode an IAP-3 homologue. To determine which of the CfMNPV IAPs is a functional inhibitor of apoptosis, we created a polyclonal cell lines constitutively expressing either IAP-1, -2, or -3. These cell lines were tested for resistance to Actinomycin D and AcMNPV induced apoptosis.

STU Poster / Viruses. V-16.

Screening of cellular factors which interact with Host Range Factor-1 (HRF-1) from *Lymantria dispar* nucleopolyhedrovirus (LdMNPV)

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HRF-1, specifically encoded by LdMNPV genome, is an essential viral factor required for productive infection of NPVs in Ld652Y cells from *L. dispar*. NPVs 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 cDNA for eukaryotic translation initiation factor, eIF3a. Nucleotide sequencing of full-length eIF3a cDNA, which was obtained by RACE method, revealed that *L. dispar* eIF3a had high amino acid sequence similarity to eIF3as from other organisms. To investigate whether HRF-1 interacted with eIF3a in Ld652Y cells, we performed 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

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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 36.894 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 SlGP41 is associated with the envelope of occlusion derived virus (ODV). Glycosylation analysis of Splt-GP41 indicated that it is not N-glycosylated. Lectin-binding assay showed that three lectins *Erythrina cristaglli* lectin (ECL), *Lycopersicon esculentum* lectin (LEL), and *Bandeiraea simlicifolia* lectin (BSL) recognizing N-acetylglucosamine were specifically bound to SlGP41. It was proposed that SlGP41 is an O-glycoprotein.

Poster / Viruses. V-18.

Molecular cloning and functional characterization of a putative glycosyltransferase family 8 member Lsp13 in *Leucania separata* multiple nuclear polyhederosis virus

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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 polyhederosis 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 experimently identify its possible functions on glycoprotein glycosylation and viral infection, we coexpressed Lsp13 with another LsMNPV glycoprotein Ld130 (F protein) and uniquely expressed Ld130 in sf9 cells, respectively. HPLC results showed Ld130 molecular is larger than its uniquely expression. We speculated that Lsp13 may changed Ld130 glycosylation and further affected virus fusion in infection. Confocal microscopy showed that a GFP-tagged P13 was only localized in the sf9 cells nuclear membrane while it located in BTI-Tn- 5B1-4(Hi5) cells both nuclear membrane and plasma mambrane. Interestingly, we found P13 expression decrease virus replication in sf9 while accelerate the Beet worm death after infection. The result suggest that P13 may play other important roles in viral replication and infection.

Poster / Viruses. V-19.

Functional analysis of FP25K of *Helicoverpa armigera* single nucleocapsid nucleopolyhedrovirus

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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 antiserum against FP25K. To study the function of HearNPV *fp25k*, a recombinant HearNPV (HaBacWD11) with an enhanced GFP gene replacing the *fp25k* was constructed using HaBacHZ8, 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 HaBacHZ8-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 HaBacHZ8-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 were the result of *fp25k* deletion. The expression of membrane fusion protein (HA133) and ODV-E66 were studied with FP25K mutant HaBacWD11 and HaBacHZ8-GFP. Surprisingly, unlike FP25K mutants in AcMNPV, which cause an increase in the expression of membrane fusion protein GP64 and a decrease of ODV-E66, no obvious changes at the expression level of Ha133 and ODV-E66 were observed in HaSNPV FP25K mutant.

Poster / Viruses. V-20.

Characterization of an AcMNPV without virions occluded in the polyhedra

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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 polyhedra was isolated. This mutant named AcDef forms normal polyhedra, but polyhedra could not infect *Trichoplusia ni* larvae *per os* at 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.

Poster / Viruses. V-21.

Analysis of the temporal expression of *Trichoplusia ni* single nucleopolyhedrovirus genes following transfection of Tn5-B1 cells

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Trichoplusia ni Single Nucleopolyhedrovirus (TnSNPV) is pathogenic to the cabbage looper (*Trichoplusia ni*), a serious pest of many economically important crops, and shows potential as biocontrol agent. TnSNPV 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 TnSNPV. 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 Tn5-B1 cells transfected with TnSNPV 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 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

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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. It shared the highest aa identity with SpliMNPV P49 (27.0%), followed by SpltNPV P49 (25.7%), suggesting that it may be a P49 homologue rather than P35 homologue. A P10 homologue was also found in choc48 as it followed the general P10 model with a coiled coil region, a proline rich region, and a basic carboxyl terminal. The PEP protein in ChocGV (*choc18*) also contained a P10-like motif present in other GVs (AdorGV, XecnGV, CrleGV). *Choc48* (P10) exhibited 30.5% aa identity with FALPE of AmEPV. Two large intergenic spaces were found between *choc21* and *choc22* (1.018kb) and between *choc22* and *choc23* (1.545kb). It was thought these regions should contain ORFs as homologues to CpGV ORFs and CrleGV ORFs expected in this area were missing. Intergenic spaces greater than 1kb are found in CrleGV and XecnGV, both corresponding to approximately the same region as the intergenic spaces in ChocGV. Phylogenetically, ChocGV was most closely related to CpGV and CrleGV.

Poster / Viruses. V-23.

The genome sequence of the *Mamestra brassicae* nucleopolyhedrovirus: An Old World virus compared with New World isolates

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We have sequenced the genome of *Mamestra brassicae* nucleopolyhedrovirus (MbMNPV). To date we have a single contig of 151820 bp and are undertaking a PCR-sequencing approach to close a gap of about 1000 bp. The full genome will be about 153000bp, which is smaller than *Mamestra configurata* NPV (MacoNPV-90/2, 155060 bp and MacoNPV-96B, 158482 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 >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 between ribonucleotide reductase 2b - and calyx-encoding genes in 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).

Poster / Viruses. V-24.

The genome sequence of *Chrysodeixis chalcites* nucleopolyhedrovirus, a baculovirus with two DNA photolyase genes

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The genome of ChchNPV, a nucleopolyhedrovirus recently isolated from *Chrysodeixis 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. ChchNPV 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 homologue in poxviruses. 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*, *ld138*, *rr1* 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 ChchNPV is uniquely protected against mutations.

Poster / Viruses. V-25.

Potential horizontal transfer of an insect trypsin-like serine protease to *Neopdiprion sertifer* NPV and *N. lecontei* NPV

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Neodiprion sertifer nucleopolyhedrovirus (NeseNPV) and *Neodiprion lecontei* NPV (NeleNPV) are the first reported baculoviruses with trypsin-like serine proteases in their genomes. Both viral proteins contained 6 conserved cysteines as well as the trypsin catalytic triad of histidine, aspartic acid and serine conserved in trypsin-like proteins from other organisms including insects and vertebrates. NeseNPV ORF 7 (*nese7*) and NeleNPV ORF 6 (*nele6*) shared top blastp matches with similar proteins from insects including Hymenoptera, Diptera, Lepidoptera, Coleoptera and Siphonaptera. Trypsin serine proteases identified in hymenopterans include those from the European hornet (*Vespa crabro*) and the honeybee (*Apis mellifera*). These hymenopteran trypsins shared higher amino acid identities with *nese7* and *nele6* than many of the hymenopteran baculovirus ORFs to lepidopteran baculovirus homologues. To determine if a horizontal transfer from the host insects to the hymenopteran baculoviruses might have occurred, trypsin genes from *Neodiprion sertifer* and *N. lecontei* were amplified by PCR, cloned, sequenced and compared to the viral genes. The nucleotide and amino acid identities of *nele6/nese7* to their host's proteins corroborated our initial hypothesis of a possible horizontal transfer from insects to the viruses. The data were further confirmed by phylogenetic analysis of viral trypsins with trypsins from other species.

Poster / Viruses. V-26.

Multitemperature single-strand conformational polymorphism - a method for detection of minute changes in baculovirus genome

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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*, *Lymantria dispar*, *Lymantria 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 excrements 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.

Poster / Viruses. V-27.

***In vivo* cloning and comparative characterization of eleven distinct entomopoxviruses isolated from sympatric populations of *Adoxophyes honmai* and *Homona magnanima* (Lepidoptera: Tortricidae)**

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Sympatric populations of *Homona magnanima* and *Adoxophyes honmai* were sampled in a tea field at Mizuho, Tokyo, Japan, to investigate the prevalence of an entomopoxvirus (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 10^6 or 10^7 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.

STU Poster / Viruses. V-28.

Searching for a homologue of the *Mythimna separata* entomopoxvirus gene encoding the protein lethal to the endoparasitoid *Cotesia kariyai*

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When the gregarious endoparasitoid *Cotesia kariyai* (Hymenoptera: Braconidae) parasitizes *Mythimna separata* (Lepidoptera: Noctuidae) larvae infected with an entomopoxvirus (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 homologues 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), *Xestia c-nigrum* GV (XecnGV), *Helicoverpa armigera* GV (HearGV), *Spodoptera litura* GV (SpltGV), *Agrotis 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, 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.

Poster / Viruses. V-29.

Promoter analysis of *Cotesia plutellae* polydnavirus and application for improved insecticides

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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.

Poster / Viruses. V-30.

Changed to oral presentation

Poster / Viruses. V-31.

Histopathological diagnosis of *Perina nuda* (Lepidoptera: Lymantriidae) infected with PnPV (*Perina nuda* picorna-like virus)

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Perina nuda picorna-like virus (PnPV) is a main pathogen of flacherie disease of *P. nuda* (Lepidoptera: Lymantriidae) larvae. PnPV is thought to persist as an inapparent infection until stress or infection of other pathogens (such as nucleopolyhedrovirus) and then causes a lethal disease of the host. The 2nd-instar *P. nuda* larvae were experimentally oral-infected with PnPV, and the infected larvae were sacrificed at the 6th-instar larval stage or newly emerged moths for detection of PnPV infection. The tissues, epidermis, muscle, nerve, fat body, tracheae, alimentary canal, gonad, silk gland, and accessary gland, were examined by RT-PCR with a PnPV-specific primer set. All examined tissues showed a PnPV-positive reaction, such results implied that PnPV tends to a systemic infection in *P. nuda*. The existence of PnPV particles in tracheae, midgut, and muscle were also confirmed by electron microscopic observation. Furthermore, an *in situ* hybridization was used to assay the tissues of the 3rd-end-instar larvae that were inoculated previously with PnPV at their 2nd-instar stage (about at 3-5 days postinoculation), and the PnPV-positive signal was only shown in the epithelial cells of the midgut. Therefore, we suggest that the epithelial cells of the midgut are initiate propagation sites of PnPV.

STU Poster / Viruses. V-32.

Modulation of GAPDH and fructose-biphosphate aldolase expression in shrimps after white spot syndrome virus (WSSV) infection

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To better understand the pathogenesis of white spot syndrome virus (WSSV), the causative agent of a serious shrimp disease, 2D gel electrophoresis was used to produce protein expression profiles from samples taken at 48-hour post infection from stomachs of specific pathogen free and WSSV-infected *Litopenaeus vanameii*. Proteins that were significantly up or down-regulated after WSSV infection were identified by in-gel trypsin digestion followed by LC-nanoESI-MS/MS and bioinformatics databases search. Two proteins that showed a marked increase in expression levels were glycoaldehyde-3-phosphate dehydrogenase (GAPDH) and fructose-bisphosphate aldolase (aldolase). Since both of these enzymes are involved in glycolysis, RT-PCR was used to determine the post infection mRNA expression levels of these and the other enzymes in the glycolysis pathway. However, only GAPDH and aldolase showed a significant increase suggesting that the up-regulation of these two enzymes is not excluding related to glycolysis. GAPDH has recently been reported to have non-glycolytic functions such as apoptosis and stress response, and can also associate with aldolase to become involved in calcium signaling. It is suggested that the altered expression levels of these two glycolytic enzymes may play important roles during WSSV infection leading to the pathogenesis of this virus-associated shrimp disease.

STU Poster / Viruses. V-33.

Identification and application of P9, the most highly expressed gene of WSSV

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White spot syndrome virus (WSSV), the type species of the genus *Whispovirus*, family *Nimaviridae*, is an enveloped, ellipsoid, large (>300kbp) 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 WSSV non-structural protein has the highest expression levels so far reported (5x higher than the major envelope protein, VP28). P9 is capable of auto-multimerization and localizes in the host cell nucleus. P9 also contains an acidic activation domain and possibly acts as a transactivater during virus infection. Apart from suggesting that P9 may play an important role during virus infection, expression of the WSSV P9 gene could be a good indicator of WSSV infection.

STU Poster / Viruses. V-34.

Identification of the nucleocapsid, tegument and envelope proteins of the shrimp white spot syndrome virus virion

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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. VP664 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*

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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 successfully transcribed in the absence of viral proteins), none 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 Sf9 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 Sf9 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^{R} - micromanipulator - a new technique for the quantitative separation of microspordian spores for infection experiments

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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 ICSI. We used an Eppendorf^{R} combination of PatchMan^{R} and CellTram^{R} devices with Eppendorf^{R} CustomTips (Type IV), attached to a Leica^{R} 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 diplocaryotic 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)

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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 otiorhynchi*, 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

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The *Nosema* disease is a worldwide honeybee epizootic disease and caused heavy economic loses 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-LSUrRNA-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 LSUrRNA 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 lepidoteran pests in Taiwan

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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 *Nosema* antiserum, a *N. bombycis*-specific primer pair and the SSUrRNA gene sequence were preliminarily detected. 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' -LSUrRNA -ITS -SSUrRNA -IGS -5S- 3'. The α- & β-tubulins and RPB1 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. plutellae*.

Poster / Microsporidia. MP-5.

Complete sequence and secondary structure of ribosomal RNA gene of the *Nosema* sp. C 01

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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 *Aphidoletes aphidomyza* (Diptera: Cecidomyiidae) as biological control agents of *Myzus persicae* (Homoptera: Aphididae)

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Intraguild interactions occur between species that are competing for similar and limited resources. Biological control organisms, such as predators or parasitoids of the same 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 *Aphidoletes aphidomyza* (Diptera: Cecidomyiidae) and *Verticillium lecanii* (Zimmermann) Viegas a predator and entomopathogenic fungus of the green peach aphid, *Myzus persicae* (Homoptera: Aphidadae). 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 *Phytoseiulus persimilis*

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Effects of using entomopathogenic fungus, *Verticillium lecanii*, against two-spotted spider mite (*Tetranychus urticae*), and its natural enemy (*Phytoseiulus persimilis*) were studied. Four isolates of *V. lecanii* (Vertalec, Mycotal, 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 spider mites, but the rate of susceptibility was different among isolates. Although, *V. lecanii* also showed pathogenicity against predatory 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 *Paecilomyces fumosoroseus*

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The survival of blastospores of *Paecilomyces fumosoroseus* during drying and storage was dependent on nutrition 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 -20C resulted in no significant loss in blastospore viability for freeze-dried preparations. When air-dried *P. fumosoroseus* blastospores were stored under vacuum at -4ºC, 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.

Poster / Microbial Control. MC-4.

Considerations in using *Metarhizium anisopliae* as a biopesticide for wireworms

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In 1999, an epizootic of *Metarhizium anisopliae* in a field population of wireworms (*Agriotes 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 10^5 condia/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 10^6 to 10^8 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*

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The fungal biocontrol agent *Beuaveria bassiana* SG8702 was bioassayed for its virulence to the eggs and female adults of carmine spider mite *Tetranychus cinnabarinus* at 25ºC 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/mm^2 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, 553 and 849 conidia/mm^2 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} estimates of 701, 597, 310, 202, 151 and 100 conidia/mm^2 on days 4-9 respectively. The LT_{50} dropped from 6.5 days at 172 conidia/mm^2 to 2.8 days at 849. An LC50 of 557 conidia/mm^2 resulted from probit analysis of the egg bioassay data. Conclusively, the fungal agent was more virulent to *T. cinnabarinus* adults than their eggs despite its high virulence to both morphs.

Poster / Microbial Control. MC-6.

Development of *Beauveria bassiana*-based mycoinsecticide for tea leafhopper control in China: Current status and prospects

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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* SG8702 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 $1x10^{10}$ or $2x10^{10}$ 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 efficacies 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

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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* SG8702 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-cm^2 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 thiosultap-diaminium insecticide on *Plutella xylostella* larvae

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Tier bioassays were performed to quantify interaction between fungal biocontrol agent *Beauveria bassiana* SG8702 and dimehypo, a thiosultap-diaminium 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/mm^2 or together with the chemical rates of 5, 10, 25, 50 and 100 μg/ml, respectively, and maintained at 25ºC 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. xylostell* with the LC_{50} being 269 conidia/mm^2 on day 4 and dropping to 107 on day 8. Lower lethal concentrations or shorter median lethal times resulted from fungal sprays including dimehypo 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 dimehypo rates of

≥25 µg/ml into the fungal sprays for LC_{50} decreases of 2.6-1756 folds, ≥50 µg/ml for LC_{70} decreases of 4-274 folds and 100 µg/ml for LC_{90} decreases of 9-33 folds. These rates were equivalent to 5-20% of the dimehypo rate labeled for field application. The fungal/chemical interaction highlights the feasibility of combined formulation or application of *B. bassiana* and dimehypo for *P. xylostella* control.

Poster / Microbial Control. MC-9.

Susceptibility of larval stages of the aphid parasitoids *Aphidius colemani* and *A. matricariae* to the entomopathogenic fungus *Beauveria bassiana*

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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 *Aphidius colemani* and *Aphidius 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*

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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 spores 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.

STU Poster / Microbial Control. MC-11.

Toxicity analysis of truncated insecticidal crystal proteins Cry1Ba3 from *Bacillus thuringiensis*

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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 *Bam*HI and *Sal*I 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 xylotella*, compared with full-length Cry1Ba3 protoxin, 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. xylotella* 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*?

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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 dose of Btk. Larvae from the reverted-susceptible population exhibited a significant 6-fold increase in resistance following exposure to the sublethal dose of Btk. This increase was not observed for the resistant and long-term, susceptible laboratory populations. Phenoloxidase activity and hemolymph protein concentration were measured for 1) naive larvae, 2) larvae 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 exhibited 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.

STU Poster / Microbial Control. MC-13.

Construction of a *Bacillus thuringiensis* BAC library and partially cloning of zwittermicin A biosynthesis cluster

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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 G03 was constructed. G03 is highly toxic to *Lepidopterans* 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 zwittermicin A biosynthesis cluster in a 1000-clone subset. Four positive clones with different inserts were obtained. The sequence of zwittermicin 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.

Poster / Microbial Control. MC-14.

Implementation of the largest worldwide laboratory production of a baculovirus: The case of the nucleopolyhedrovirus of *Anticarsia gemmatalis* (Lep.: Noctuidae) in Brazil

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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 transfered 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 aproximately 1.4 million hectares of soybean/year.

Poster / Microbial Control. MC-15.

Laboratory and orchard studies on the transmission of *Cydia pomonella* granulovirus by contaminated *C. pomonella* adults

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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.

NEMATODES

Poster / Nematodes. N-1.

Evaluation of a native *Heterorhabditis* species from the Coastal Region of Central Perú against white grubs

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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 (*Bothynus 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 LC_{50} 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/cm^2 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 *Symmetrischema tangolias*).

Poster / Nematodes. N-2.

Targeting the Andean weevils with a native entomopathogenic nematode species

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An entomopathogenic nematode in the genus *Heterorhabditis*, designated as Alcazar-1, was isolated from potato weevil (*Premnotrypes suturicallus*) 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.

Poster / Nematodes. N-3.

Virulence of various commercial isolates of *Heterorhabditis bacteriophora* against the European chafer (*Rhizotrogus majalis*)

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The European chafer (EC) is the most damageable 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-cm^3 plastic Solo cups at 24ºC. 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

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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 Mg^{2+} and $Mn^{2+}$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 juvenile yields 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 Mn^{2+} 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.

STU Poster / Nematodes. N-5.

Infectivity of entomopathogenic nematodes and immune responses of their insect hosts

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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 chafer larvae *Cyclocephala borealis*, and house cricket *Acheta domesticus*. 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. Interesting, the *S. glaseri* NC strain has stronger pathogenity compare to the *S. glaseri* FL strain in the same hosts. Northern masked chafer 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

Poster / Other. O-1.

The *Sleeping Beauty* transformation system: A new approach for the study of tick cell microbe interactions

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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 *Tc1/mariner* related transposable element and a reconstructed transposase from teleost fish sequences (Ivics et al. 1997 Cell 91:501), to mediate transformation of ixodid tick cells. Cell line ISE6 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.

SYMPOSIUM (Cross-Divisional). Wednesday, 4:30–6:30

Molecular interactions between insect vectors and human pathogens

Symposium. Wednesday, 4:30. 143

Molecular interactions between the malaria parasite and its mosquito vector

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Malaria is one of the deadliest infectious diseases that kills about 2 million persons every year. New strategies to contain transmission are urgently needed and interruption of the parasite cycle in the mosquito (the obligatory vector) is an option that needs to be explored. Strong circumstantial evidence indicates that molecular recognition is a requisite for sporozoite invasion of the mosquito salivary gland, but information on this subject is scant. Using a phage display library we have previously identified SM1, a dodecapeptide

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 saglin) 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. 144

Relationships between the symbiont *Sodalis glossinidius* and the vectorial competence of tsetse flies

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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 gambiensis* 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. gambiensis* 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. 145

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 apocomplexan 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 acetylate 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. 146

Sand fly midgut receptors for *Leishmania* parasites: Targets for transmission-blocking vaccines

Shaden Kamhawi[2], Marcelo Ramalho-Ortigao[1], Van M. Pham[1], Sanjeev Kumar[3], Phillip G. Lawyer[2], Salvatore J. Turco[4], Carolina Barillas-Mury[3], David L. Sacks[2], Jesus G. Valenzuela[1]

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We have isolated from a *P. papatasi* sand fly midgut cDNA library a transcript coding for a galectin (PpGalec). The observed homology of PpGalec to galactose-binding proteins, together with previous studies indicating that poly-Gal (β1-3) 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 (β1-3) 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.

CONTRIBUTED PAPERS. Wednesday, 4:30-6:30

MICROBIAL CONTROL 2

Contributed paper. Wednesday, 4:30. 147

Interactions between the granulovirus *Po*GV and *Bacillus thuringiensis* (Berliner) against the potato tuber moth, *Phthorimaea operculella* (Zeller)

Marc Sporleder[1], Dante Mamani[2], Jürg Huber[3], Jürgen Kroschel[1]

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The potato tuber moth (PTM), *Phthorimaea operculella* Zeller (Lepidoptera: Gelechiidae), is a major pest of potatoes in many tropical and subtropical regions. The larvae mine leaves and tubers, causing damage to potatoes in storage and in the field. In stores, the pest can be efficiently controlled by using the endemic granulovirus (*Po*GV). That is why *Po*GV has become an integral part of IPM approaches. In the field, host populations may be successfully suppressed by using *Po*GV, but due to the high amount of infected larvae needed, its use in the field seems unfeasible. One strategy to enhance the eficacy of baculoviruses could be application with a synergist. In order to promote bio-pesticides as components of IPM for potato production, the objective of the present study was to determine the mode of interaction between *Po*GV and spore-endotoxin of *Bacillus thuringiensis* var. *kurstaki* (Javelin, a commercial preparation) (*Bt*). The concentration-mortality response of 1st instar *P. operculella* larvae due to *Po*GV and to *Bt*, using five concentrations each, and to simultaneous applications of both agents in different combinations (that is, 3:1, 1:1, 1:3) using the same concentrations, was assessed in the laboratory. Chi square and a

binominal test were used to compare observed and expected percentage mortalities of the different mixtures to test three different hypotheses for the mode of action of a given mixture (that is, synergism, additive mode of action, and antagonism). The results showed that the interaction between *Po*GV and *Bt* was mixed, but tended towards antagonism. Synergistic effects were only discernible when the larvae were exposed to low concentrations of both pathogens. That is why simultaneous application of *Po*GV and Bt seems not to be a feasible approach for PTM control.

Contributed paper. Wednesday, 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

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Granulovirus targets larvae before or during initial entry into fruit and provides growers with an option for codling moth (CM) control that is 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/ac) and spray intervals (7, 10, or 14 days) in an experimental orchard and to compare different rates of virus (1, 2, or 3 oz/ac) applied weekly to Guthion in a conventionally managed orchard heavily infested with CM. Virus applications did not reduce fruit damaged by CM, but there were significantly fewer deep entries and surviving larvae among virus-treated fruit. The vast majority of damage was in the form of shallow stings and larval mortality was consistently high. Higher doses and shorter application intervals resulted in consistently fewer deep entries and higher mortality rates. In the ½ acre commercial plots treated with virus, there was less CM damage compared with untreated areas, but more compared with Guthion-treated areas. Rates of CM mortality in virus-treated plots were similar to those observed in individual trees sprayed with equivalent rates of virus in the previous study. Data from interception traps showed far fewer moths in virus-treated and Guthion-treated plots compared with untreated areas. The dosage and application frequency of virus that provides acceptable control will depend largely on the localized pressure of codling moth.

Contributed paper. Wednesday, 5:00. 149

Semiochemical driven auto-dissemination of viruses for the control of orchard pests

D. Winstanley[1], J. V. Cross[2], N. Naish[1], G. Keane[1], S. Hilton[1], D. Gajek[2], R. van Wezel[2]

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Defra are funding a three year project to develop a novel strategy for the control of two tortricid orchard pests (codling moth, *Cydia pomonella* and summer fruit tortrix, *Adoxophyes orana*), using contaminated adult moths to disseminate pathogenic viruses in the orchard. The project is being carried out in the UK by Warwick HRI, in conjunction with EMR at East Malling. *Cydia pomonella* granulovirus (CpGV) and *Adoxophyes orana* granulovirus (AdorGV) are highly pathogenic to their target host and will be used in this project. Both viruses are used in sprayable biopesticides in Europe, with considerable success. The moths are contaminated in virus-charged pheromone dispensers in the orchard. These are fitted with a lure that emits either a pear-derived volatile with pheromonal potency (Ethyl (2E, 4Z)-2, 4-decadienoate, DA), that attracts both male and female codling moth, or sex pheromones that attract either male summer fruit tortrix or codling moth. In the first two years, two virus formulations were assessed for their ability to be picked up in the orchard and disseminated from a central tree in one hectare plots. In addition, orchard experiments were conducted to develop the optimum dispenser design. A summary of the first two years results will be presented.

Contributed paper. Wednesday, 5:15. 150

Biotic and abiotic factors affecting the field persistence and residual efficacy of *Cryptophlebia leucotreta* granulovirus on citrus

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False codling moth, *Cryptophlebia leucotreta* (Meyrick) (Lepidoptera: Olethreutidae), is a fruit pest of citrus, macadamias, stone fruits, avocadoes and litchis, in southern Africa. Chemical control of *C. leucotreta* is problematic for a number of reasons. Recently, a commercial formulation of a South African isolate of the *Cryptophlebia leucotreta* granulovirus (CrleGV-SA) was registered under the name, CRYPTOGRAN, for use on citrus in South Africa. Semi-commercial field trials on navel orange trees revealed that *C. leucotreta* infestation of fruit was significantly reduced for up to 17 weeks after application of CRYPTOGRAN. This length of control was unexpected, as a previous trial with the virus had shown that within 3-6 days after application, original activity remaining (OAR) on fruit directly exposed to sunlight, was reduced to below 50%. A number of possible reasons for this apparent protracted persistence of CrleGV will be discussed. These are: protection of the virus provided by the shade of the tree canopy; protection provided by the navel end of the fruit, where the majority of larvae enter the fruit; rainfastness of the virus; horizontal transmission of the virus; the slow rate of recolonisation of orchards by *C. leucotreta*; and the compatibility of the product with *C. leucotreta* parasitoids.

Contributed paper. Wednesday, 5:30. 151

Can pathogens be used for eradication of soil pests?

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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 and are considered difficult targets 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.

Contributed paper. Wednesday, 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).

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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 *H. 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. 153

Combined use of insect pathogenic fungi and nematodes against the onion thrips, *Thrips tabaci* in the field

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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

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Pseudomonas fluorescens is an important biocontrol bacterium for agriculture. The engineered bacterium, Biop8 that *P. fluorescens* P303 was obtained with *cry1Ab, cry1Ac* and *cry2Aa* from *Bacillus thuringiensis* in two different kinds of shuttle plasmid vectors, is toxin active against insects and supresses 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 stains 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 exceeds 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.

CONTRIBUTED PAPERS. Wednesday, 4:30-6:30

VIRUSES 3

Contributed paper. Wednesday, 4:30. 155

Specific binding of AcMNPV ODV to midgut cellular targets is mediated by *pif* genes *Ac022* and *Ac119*, but not *Ac115*

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Per os infectivity factor (*pif*) genes are essential for baculovirus occlusion-derived virus (ODV) to infect insect hosts orally. AcMNPV *pif* genes include *Ac022*, *Ac119* and *Ac115* which are widely conserved among baculoviruses. We used a fluorescence dequenching assay to determine whether the products of these AcMNPV *pif* genes are important in specific binding and/or fusion of ODV to midgut cellular targets. We generated deletion mutants for each *pif* gene by inserting the *hsp70/lacZ* cassette into their respective ORFs. We then compared binding, fusion and competition of each *pif* mutant with wild-type ODV in *Heliothis virescens* larvae. Binding and fusion of ODV lacking either *Ac022* or *Ac119* was significantly reduced (~65%) relative to wild-type ODV and qualitatively different. By contrast, ODV lacking *Ac115* bound and fused at levels equivalent to wild-type virus. ODV of the *Ac115*, but not the *Ac022* or *Ac119* deletion mutants, competed progressively with wild-type ODV, indicating that *Ac115* was not required for specific ODV binding to midgut cellular receptors, while the other two were. Our results support the conclusion that *Ac022* and *Ac119* are ODV attachment proteins that bind to specific receptors on midgut epithelial cells, whereas Ac115 is required for some other function.

STU Contributed paper. Wednesday, 4:45. 156

Impact of the peritrophic matrix on baculoviral pathogenesis in a tritrophic system

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In lepidopteran larvae, the success of a baculovirus infection can be reduced by interactions among the larva, the virus and the diet consumed. Here, we examined the effect of ingested cotton foliage on baculoviral pathogenesis in *Heliothis virescens*. Larvae fed cotton foliage immediately before inoculation with *Autographa californica* nucleopolyhedrovirus (AcNPV) suffer significantly less viral mortality than larvae fed artificial diet. Enhancin, a metalloprotease found in granules of the *Trichoplusia ni* granulovirus (TnGV) that disrupts the peritrophic matrix (PM) of some lepidopteran larvae, was used to elucidate the role of the PM in this cotton-mediated suppression of mortality. A lacZ-expressing construct of AcNPV was used to compare viral pathogenesis between cotton-fed *H. virescens* larvae in the presence and absence of enhancin prior to AcNPV inoculation. Larvae exposed to enhancin exhibited a greater number of primary midgut infections and a higher final viral mortality than larvae that did not receive enhancin. This finding suggests that inhibition of fatal infection in cotton-fed *H. virescens* challenged with AcNPV involves plant-mediated changes in the permeability of the PM to virions. This work provides further insight into the basic interactions between insects, their host plants and pathogens that infect them; moreover, a better understanding of these interactions may improve insect control strategies.

STU Contributed paper. Wednesday, 5:00. 157

The effect of tannic acid on gypsy moth performance and susceptibility to the nuclear polyhedrosis virus

Viatcheslav V. Martemyanov, Zhana O. Markina, Sergej A. Romancev, Stanislav A. Bahvalov

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The effect of tannic acid, which is one of the chemicals involved in the immune response of woody plants caused by defoliation, on the

performance of gypsy moth (*Lymantria dispar* L.) and its susceptibility to nuclear polyhedrosis virus (LdNPV) was studied. The following parameters were estimated: larvae weight, mortality rate, activities of detoxification (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

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The *Autographa californica* nucleopolyhedrovirus (Ac*M*NPV) encodes a gene (open reading frame 32) with homology to vertebrate and invertebrate fibroblast growth factors (*fgfs*), key regulators of developmental processes affecting the growth, differentiation, and migration of many cell types. We studied the temporal regulation of the Ac*M*NPV *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 NeabNPV-infection in balsam fir sawfly, *Neodiprion abietis* larvae.

Beatrixe Whittome[1], Benoit Morin[2], Christopher Lucarotti[2], Dan Quiring[3], and David Levin[1]

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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* nulceopolyhedrovirus (NeabNPV) in virus-infected larvae of the Balsam fir sawfly. Second and third instar larvae of *N. abietis* were infected with 1000 NeabNPV 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 progenerative 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 Nie[1,2], Qian Wang[1,3], Changyong Liang[1,3], Zehua Yu[2] and Xinwen Chen[1]

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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 nucleocytoplasmic 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 Rhordamine-Phalloidin, HA2 was found to colocalize with actin.

Contributed paper. Wednesday, 6:00. 161

Co-opting actin and the Arp2/3 complex for baculovirus progeny production

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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 Syndrom 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, in produced late and shows homology to WASP. Moreover, 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.

Contributed paper. Wednesday, 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

Xiaojiang Dai[1,2], Basil M. Arif[3], Peter J. Krell[2], and David A. Theilmann[1]

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Exon0 (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 we examined whether *exon0* homologues from heterologous baculoviruses can complement an AcMNPV *exon0* knockout mutant by rescuing budded virus production. AcMNPV *exon0* was knocked out using recombination in an infectious bacmid. *Exon0* homologues from five heterologous baculoviruses were co-transfected with the AcMNPV *exon0* knockout. This included two Group I NPVs, *Choristoneura fumiferana* MNPV, *Orgyia pseudotsugata* MNPV, and three Group II NPVs, *Trichoplusia ni* Single NPV, *Mamestra configurata (Maco)* NPV-A, and *Maco*NPV-B. The *exon0* homologous genes were HA-tagged and placed under control of the AcMNPV *exon0* late promoter. Initial results showed that all the EXON0 homologues from the heterologous NPVs were able to rescue the AcMNPV *exon0* knockout for budded virus production. However, *exon0*-knockout viruses with heterologous *exon0s* did not spread as efficiently as wild-type virus and it appeared that production of budded virus was also delayed. This suggests EXON0 contains virus specific determinants required for budded virus production.

Workshop (Microbial Control Div). Wednesday, 8:30-9:30

Bioassays with insect pathogens: Concepts and performance

Workshop paper. Wednesday, 8:30. 163

Methods for analysis of data from bioassays with insect pathogens

James E. Throne

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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 replications and number of individuals to test per replication (and what to do with replications 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.

THURSDAY - 11 August

SYMPOSIUM (Div. of Microsporidia). Thursday, 8:00-10:00

Why study Microsporidia? Interesting research areas besides taxonomy and biological control

Symposium. Thursday, 8:00. 164

Some effects of compaction on microsporidian nuclear genomes

Bryony Williams and Patrick J. Keeling

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The nuclear genome is typically quite spacious, but it has been highly compacted in several cases, most spectacularly in microsporidian parasites. We have conducted genome sequence survey (GSS) and expressed sequence tag (EST) surveys to study effects of this compaction, and two will be discussed here. Mitochondrial proteins are targeted using an N-terminal transit peptide, but we have found that most microsporidian mitochondrial proteins lack such extensions. Nevertheless, using GFP-fusion proteins in yeast we show that microsporidia appear to use a homologous but simplified system of targeting, perhaps using the N-terminus but not as a leader. Gene expression has been studied 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 polycistronic: 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.

Symposium. Thursday, 8:30. 165

Microsporidian parasites of crustacea, specificity, sex and populations

Judith E. Smith[1], Johanna G. M. Slothouber Galbreath[2], Yang Qui[1], James J. Becnel[3] and Alison M. Dunn[1]

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The Microspora are an ancient and divergent group of parasites that infect all phyla. Current molecular phylogenetic analysis suggests that a major radiation of these parasites may have occurred in the crustacea and they are particularly common in amphipods with more than 20 novel species discovered from 18 host species. In studies of UK amphipods we have demonstrated that microsporidian parasites have great importance. In these systems vertical transmission is common and although parasites rarely cause gross pathology they exert a powerful influence on their hosts by disrupting sexual differentiation and causing sex ratio distortion. We have evidence that vertically transmitted microsporidia have been retained during transcontinental invasion of their amphipod hosts and propose that parasitic sex ratio distortion may facilitate host invasion by increasing the reproductive rate of the invading host species. The impact of these parasites on host sex determination, population growth and stability, biological invasion and community structure within amphipods calls for a wider evaluation of their role in crustacean hosts.

Symposium. Thursday, 9:00. 166

Epizootiology of *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

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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 polygyne (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 polygyne 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 polygyne ants disappeared. Long-term epizootics developed when the microsporidium was released in two predominantly polygyne populations but not at two monogyne sites. In mixed host populations, prevalence peaked at

>75% in both social forms; the form suffering higher prevalence decreased proportionally to the other. Prevalence averaged 47-57% and did not vary seasonally. The microsporidian rate of spread was 0.8-9.4 m/month. *T. solenopsae* weakened ant populations only sporadically, through decreases in numbers of foragers, colony numbers, colony size, or brood.

Symposium. Thursday, 9:30. 167

The genus *Brachiola* and human skeletal muscle infection caused by the mosquito microsporidium, *B. algerae*

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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 plasmalemma, 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 plasmalemmal 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 electron microscopic 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 cause myositis and ultimately lead to the death of a 57- year-old woman.

CONTRIBUTED PAPERS. Thursday, 8:00-10:00

VIRUSES 4

Contributed paper. Thursday, 8:00. 168

Establishment of a natural phylogeny of Baculoviruses

Rüdiger Hauschild[1], Martin Lange[1], Olaf Bininda-Emonds[2], Johannes A. Jehle[1]

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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 familiy 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. Jakubowska[1,2], René M. Klein Lankhorst[3], Jadwiga Ziemnicka[1], Just M. Vlak[2] and Monique M. van Oers[2]

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The turnip 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 (*pif-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 GVs infecting the same host.

Contributed paper. Thursday, 8:30. 170

Genome sequence and organization of the *Neodiprion abietis* nucleopolyhedrovirus.

Simon Duffy[1], Benoit Morin[2], Christopher Lucarotti[2], and David Levin[1]

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The *Neodiprion abietis* nucleopolyhedrovirus (NeabNPV) 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 pathology. Like the *N. lecontei* and *N. sertifer* NPVs (NeleNPV and NeseNPV, respectively), NeabNPV has a relatively small genome (85.7Kb) 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 NeleNPV 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. 171

Morphological, molecular, and genomic characterization of two mosquito Cypoviruses

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The morphological, genomic, and molecular characteristics of two cypoviruses (cyptoplasmic polyhedrosis virus, CPV) from the mosquitoes *Culex 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 TnCPV-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 lepidopterian hosts.

Contributed paper. Thursday, 9:00. 172

Integration of an ichnovirus genome segment in the genomic DNA of lepidopteran cells

Daniel Doucet[1], Anic Levasseur[1], Catherine Béliveau[1], Don Stoltz[2] and Michel Cusson[1]

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At the time of parasitization, the ichneumonid *Tranosema rostrale* injects a polydnavirus that assists the wasp egg and larva in subjugating the development and immunity of its host, the spruce budworm (*Choristoneura fumiferana*). *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 examined. We wanted to test the hypothesis that the integration of segment F in the genomic DNA of Cf-124T cells is responsible for the sustained expression. A Southern analysis of DNA isolated from TrIV-inoculated Cf-124T cells showed that episomal copies of segment F disappear four days after TrIV inoculation, while a high-molecular form persists for more than 100 days. This suggested an intimate association between Cf-124T genomic DNA and segment F. 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. 173

Comparison of genome organization and encoded proteins in campoplegine and banchine ichnoviruses

Renée Lapointe[1], Bruce A. Webb[2], Kohjiro Tanaka[2], Walter Barney[2], Don Stoltz[3] and Michel Cusson[1]

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Natural populations of the spruce budworm, *Choristoneura fumiferana*, are regulated by various natural enemies, including the ichneumonid wasps *Tranosema rostrale* (subfamily Campopleginae) and *Glypta fumiferana* (subfamily Banchinae). These endoparasitoids oviposit at different times of their host's life cycle: *G. 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 ichneumonid (*Ichnovirus*; IV) and braconid (*Bracovirus*; BV) wasps indicate that the two groups differ with respect to gene content and families. Here, we show that among IVs, differences are also observed between the virus of the campoplegine *T. rostrale* (TrIV) and that of 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. 174

Display of a foreign protein using recombinant baculovirus occlusion bodies: A novel vaccination tool

Rebecca Wilson[1], YeonHo Je[1], Laurence Bugeon[1], Ursula Straschil[1], David R. O'Reilly[1] and Julie A. Olszewski[1,2]

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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. Recombinant 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 King[1], Kevin Richards[1], Richard Hitchman[1], Helen Irving[1], Susan Mann[1], Evi Siaterli[1] and Robert Possee[2]

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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, *flash*BAC 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, recombination 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 baculovius 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 Liu[1] and Leah S. Bauer[1,2]

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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 eradiction 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 Knight[1,2], Caroline Hauxwell[1], David Holdom[1], Gordon Simpson[3]

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Mirids (*Creontiades* 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 pule 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. Dara[1], Michael R. McGuire[2] and Harry K. Kaya[3]

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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. McGuire[1] and Jarrod E. Leland[2]

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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 10^{13} conidia/acre in June and 10^{13} 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.

Contributed paper. Thursday, 9:00. 180

Selection and field evaluation of *Beauveria bassiana* isolates for control of tarnished plant bug, *Lygus lineolaris*

Jarrod E. Leland[1], Michael R. McGuire[2], and Jeff Gore[1]

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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 mycoinsecticide development. Evaluation criteria included; pathogenicity to *L. lineolaris* and *L. hesperus*, impact on beneficial insects, spore production, mycotoxin 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.

Contributed paper. Thursday, 9:15. 181

Fungal BCAs: Potential control agents to control subterranean pests

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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 horticola*), a number of different larvae of Elateridae (e.g. *Agriotes obscurus, Bothynoderes punctiventris, Otiorhynchus 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 fulfil the key criteria of BCAs by effectiveness, autodissemination and their excellent persistence. This paper provides examples of the successful use of the entomopathogenic Hyphomycetes *Beauveria* and *Metarhizium* in subterranean pest control in European agriculture, forestry and horticulture. Preventive control approaches based on the subterreanean pests listed above will be discussed.

STU Contributed paper. Thursday, 9:30. 182

To germinate or not? Strategies of *Beauveria bassiana* for survival in soil

Carolyn V. Mander[1], Trevor A. Jackson[2] and Bruce Chapman[1]

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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.

STU Contributed paper. Thursday, 9:45. 183

***Metarhizium anisopliae* conidia produced under environmental and nutritional stresses exhibit increased virulence and tolerance to UV-B radiation and heat**

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Fungi constantly adapt their physiology in response to the environmental stresses they encounter, and they thereby often develop tolerance to previous debilitating stresses. In this study, we subjected *Metarhizium anisopliae* var. *anisopliae* (ARSEF 2575) to various stress conditions during growth, and subsequently tested the conidia so produced for changes in tolerance to ultraviolet radiation and heat. Also, we assayed for changes in virulence to *Tenebrio molitor* larvae. Growing the fungus under nutritive stress [minimal medium (MM) = Czapek medium without saccharose or MM plus 3% lactose (MML)] produced conidia with UV-B-radiation tolerance twice that of conidia produced on potato dextrose agar plus yeast extract medium (PDAY). Heat tolerance, however, increased only ca. 20% when conidia were produced on MM rather than PDAY. Conidia were most virulent when produced on MML (LT_{50} = 2.6 days), followed by MM (2.8 d), and PDAY (3.6 d). Conidia produced on MML had the highest hydrophobicity (which increased attachment of conidia to host cuticle) and earliest germination, and this combination probably enhanced virulence. When the fungus was grown on PDAY medium amended with NaCl or KCl to increase osmolality, the UV-B tolerance increased proportionally with increases in salt concentration. At concentration of 0.8 M for both of these salts, the UV-B tolerance doubled that of PDAY alone. Conidia produced on medium with 0.4 M NaCl or KCl had twice the heat tolerance of those produced on PDAY. Thermotolerance, on the other hand, decreased as salt concentration increased. Conidia produced under osmotic stress were more virulent than conidia from PDAY [LT_{50} (NaCl or KCl 0.8 M) = 3.0 d], but virulence decreased when conidia were produced on 0.6 M NaCl or KCl, (LT_{50} = 3.5 and 3.4 d, respectively). Growing the fungus under oxidative stress by amending the PDAY medium with 5 mM H_2O_2, did not alter the conidial UV-B or heat tolerance nor virulence (LT_{50} = 3.5 d) in comparison with conidia produced on PDAY medium. Therefore, the highest phenotypic plasticity for stress tolerance and virulence was observed when the fungus was grown under nutritive stress. Conidial yield, however, was somewhat too severely reduced by all of the nutritive and osmotic stress during growth. Oxidative stress (H_2O_2) did not alter spore production. We conclude that perseverance and virulence can be enhanced by manipulation of fungal culture conditions.

CONTRIBUTED PAPERS. Thursday 8:00-10:00

BACTERIA 3

Contributed paper. Thursday, 8:00. 184

Variability of fitness costs associated with Cry1A resistance in *Helicoverpa armigera* on cotton and alternative refuge crops

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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 F_1 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 Meunier[1], Gabrielle Préfontaine[1],Qili Feng[2], Roland Brousseau[1] and Luke Masson[1]

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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 Cry 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. Gassmann[1], S. Patricia Stock[2], Yves Carrière[1], and Bruce E. Tabashnik[1]

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We evaluated effects of entomopathogenic nematodes on the fitness cost of resistance to Bt toxin Cry1Ac in the pink bollworm, *Pectinophora gossypiella* (Lepidoptera: Gelechiidae). *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

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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 equaled 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)

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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 dt. 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* subsp. *toguchini* in environment

Viktor P. Khodirev

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The search of various strain of *Bacillus thuringiensis ssp. toguchini H31* (*Bt H31*) was realized in different types of soil, bran and dead insect. *Bt H31* was found in chernozem ($1\text{-}10^4$ spore/1g dried soil) and many other types of soil in Novosibirsk region. This bacterium

was found in the adults of beetles *Melasoma lapponica* L. and in the larvae *Chrysomella lapponica* L. *Bt H31* was isolated from different bran and there was a good graining and large crystals in bacteria. In general 105 strains of *Bt H31* was determined in different substrates. The inoculation with *Bt H31* resulted to increase of mortality of larvae *Leptinotarsa decemlineata* Say. (50-70%), *Tenebrio molitor* (20%) and *Hyponomeuta evonymellus* L. (100%) in comparison with control. The parasporal crystals *Bt H31* strain contained two major proteins with masses 65, 40 kDt and minor proteins with masses 120, 130, 90, 75 kDt.

STU Contributed paper. Thursday, 9:30. 190

Targeted delivery of genetically conjugated Cyt1Aa from *Bacillus thuringiensis* subsp. *israelensis* into myeloma model cells

Shmuel Cohen[1,2], Eitan Ben-Dov[1], Marina Nisnevitch[2], Rivka Cahan[2], Michael Firer[2] and Arieh Zaritsky[1]

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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 (VHFFKNIVTPRT), 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, 9:45. 191

Differential expression of cry toxin in a *Bacillus thuringiensis* strain with dual insecticidal activity

Javier Torres[1], Norma A. Valdez-Cruz[1], Jose D. Tinoco[2], Sergio Orduz[1,3]

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Transcription of *cry* genes is carried out during sporulation and is directed by sporulation transcription σ 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 increase 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, was observed a critical point after the Cry1 transcription (on study) during vegetative grown, because discordance between mRNA and protein levels was found.

SYMPOSIUM (Division of Bacteria). Thursday, 1:30–3:30

Toxin-receptor interactions and mode of action

Symposium. Thursday, 1:30. 192

Influence of the physico-chemical and biochemical environment on the kinetics of pore formation by Cry toxins

Vincent Vachon, Jean-Louis Schwartz and Raynald Laprade

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 Adang[1,2], Gang Hua[1], Jiang Chen[1], Juan Luis Jurat-Fuentes[1], and Mohd Amir Abdullah[1]

Departments of Entomology[1] and Biochemistry & Molecular Biology[2], 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 glycosylphosphatidyl inositol 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.

Symposium. Thursday, 2:30. 194

Structural and functional analysis of the pre-pore and membrane inserted pore of Cry1A toxins.

Liliana Pardo[1], Isabel Gómez[1], Carlos Muñoz-Garay[1], Nuria Jimenez-Juaréz[1], Sarjeet S. Gill[2], Mario Soberón[1] and Alejandra Bravo[1]

[1]Instituto de Biotecnología Universidad Nacional Autonoma de Mexico, Cuernavaca 62250 Mor. Mexico, [2]Department of Cell Biology and Neuroscience, University of California, Riverside, CA92521, USA

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 Cry1 toxins with its target membrane.

Symposium. Thursday, 3:00. 195

Toxin binding site of the *Heliothis virescens* cadherin

Ruiyu Xie[1], Meibao Zhuang[1], Linda S. Ross[1], Isabel Gomez[2], Karlygash Aimanova[1], Daniela I. Oltean[1], Alejandra Bravo[2], Mario Soberón[2] and Sarjeet S. Gill[1]

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Bacillus thuringiensis Cry1A proteins exerts their toxic effect through a receptor-mediated process, 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 antiserum 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 midgut 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 (Div. of Nematodes). Thursday, 1:30-3:30

Ecology of entomopathogenic nematodes

Symposium. Thursday, 1:30. 196

Biogeographic distribution and diversity of entomopathogenic nematodes: Natural patterns or human- biased trends?

S. Patricia Stock

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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

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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

E.E. Lewis[1], E.E. Perez[2], H. Arimoto[1], J.F. Campbell[3], D.I. Shapiro-Ilan[4]

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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 (IJs) 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 I. Shapiro-Ilan

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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

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A wide variety of abiotic factors can affect the ability of infective juveniles (IJs) 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 with 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

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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, agrichemical 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

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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

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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.

CONTRIBUTED PAPERS. Thursday 1:30-3:30

VIRUSES 5

Contributed paper. Thursday, 1:30. 204

On the analogy of the baculovirus and whispovirus DNA binding proteins

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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 aspecific, 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* (Se)MNPV *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 phosporylation sites, phosporylated 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. Erlandson[1], Taryn M. Stewart[3], Leslie G. Willis[2], and David A. Theilmann[2,3]

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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 IE1 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 net work for the activation of early promoters through baculovirus polyhedrin upstream sequence

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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 (Lo et al., 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 Se*M*NPV late gene transcription and genome replication factors in the insect cell line SF-21

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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 Ac*M*NPV late expression factors (*lefs*) 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 (Se*M*NPV) epitope-tagged homologs of the Ac*M*NPV *lefs* under control of the constitutive *Drosophila* heat shock 70 protein promoter and tested their ability to activate an *Autographa californica* NPV (Ac*M*NPV) late promoter-reporter gene cassette in SF-21 cells. We tested the potential of individual or predicted functional groups of BmNPV or Se*M*NPV *lef(s)* to successfully replace the corresponding Ac*M*NPV gene(s) in transient late gene expression assays. We found that most, but not all, BmNPV *lefs* were able to fully or partially substitute for the corresponding Ac*M*NPV homolog in the context of the remaining Ac*M*NPV *lefs*. However, only the Se*M*NPV *lef-5* was able to substitute for the Ac*M*NPV *lef-5* in this functional assay. Finally, we also tested the ability of BmNPV, Se*M*NPV, and Ac*M*NPV 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. Clem[1], Louis Heaton[1], and Miriam Burton[2]

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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. 209

Identification and functional analysis of *Leucania separata* multiple nuclear polyhedrosis virus *iap3* and *p49* genes in sf9 cells

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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 *opiap3* (44%), while *ls-p49* was only 28% homology with reported *sl-p49*. *Ls-p49* also contained a reactive-site loop (RSL), while had different predicted cleavage site (KKLD74 G or SATD87 E) from *sl-p49* (TVTD94 G). Functional analysis of *ls-iap3* and *ls-p49* by transient expression assay in Sf9 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 sf9 cells. However, whether *ls-iap3* or *ls-p49* or both with anti-apoptotic functions in Sl cell is under-studying.

Contributed paper. Thursday, 3:00. 210

Characterization of a novel entomopoxvirus homolog of baculovirus P35

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We have identified a gene from *Amsacta 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 previously unknown function. 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 100-fold excess of P33 was used. Similar to P35, P33 was cleaved by DrICE and the cleavage fragments stably associated with DrICE. When *AMVp33* was silenced using RNAi during AmEPV infection of LD652Y cells, apoptosis was observed that was not observed in control treated cells. Thus,*AMVp33* encodes a substrate inhibitor similar to baculovirus P35 with a preference for effector caspases that is required to inhibit apoptosis during AmEPV infection of Ld652Y cells.

Contributed paper. Thursday, 3:15. 211

ORF390 of white spot syndrome virus genome is identified as a novel anti-apoptosis gene

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Many viruses contain the antiapoptotic genes to block the defense-by-death response of host cells. In this study, we tried to identify the putative antiapoptotic genes in white spot syndrome virus (WSSV) genome. Firstly, we confirmed that the apoptosis of shrimp primary cells triggered by actinomycin D was inhibited by WSSV infection. To identify the putative apoptotic suppressor gene of WSSV, overlapping cosmid clones representing the entire WSSV genome were individually cotransfected along with AcMNPVΔp35k/pol+ DNA. By the marker rescue assay and further sequence analysis, the ORF390 was identified as a novel antiapoptotic gene, which displays two putative caspase9 cleavage sites LLVETDGPS, VKLEHDGSK, a caspase3 cleavage site EEDEVDGVP. The Sf9 cells transfected with the recombinant vector pIE1-ORF390 did not showed obvious characteristics of apoptosis when infected with AcMNPVΔp35k/pol+. And the transient ORF390 expression allowed AcMNPVΔp35k/pol+ replication in Sf9 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.

SYMPOSIUM (Div. of Viruses). Thursday, 4:00-6:00

Insect expression systems, gene therapy and vaccine development

Symposium. Thursday, 4:00. 212

Protein N-glycosylation in the baculovirus-insect cell system.

Donald L. Jarvis, Jared J. Aumiller, and Jason R. Hollister

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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 paucimannose 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. 213

Densovirus-derived vectors for stable expression of foreign proteins in insect cells and somatic transformation of insects

Max Bergoin

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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 eythropoïetin and human γ interferon. Sf9 cell clones constitutively expressing these genes were obtained and the biological activity of recombinant proteins was demonstrated. By microinjecting *Drosophila melanogaste*r 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 tool for somatic transgenesis. Taken together these results demonstrate the flexibility and reliability of using densovirus-derived vectors for multiple biological applications.

Symposium. Thursday, 5:00. 214

BacMam viruses: Versatile tools for mammalian cell-based assay development

J. Patrick Condreay

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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 transduced 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

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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 *Theileria 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 GFP or to the baculovirus envelope glycoprotein GP64, resulting either in cytoplasmic expression or surface display. In this way, stable and more-authentic p67 antigens were obtained. By testing these antigens in different animal models highly immunogenic vaccine candidates were obtained, which were efficacious against ECF in cattle at much lower doses than with previously produced recombinant p67 and with a single boost immunization. By replacing the p67 signal peptide with the honeybee mellitin signal surface and by removing its transmembrane domain, a secreted form was obtained. The integrity of this secreted protein was further improved 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 PAPERS. Thursday, 4:00-4:45

BACTERIA 4

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

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Bacillus sphaericus strains may produce the highly potent mosquitocidal 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 IAB59 and will report their characterisation and possible role in mosquitocidal activity.

1) Appl. Environ. Microbiol. 68: 3003-3009 (2002); 2) Med. Vet. Entomol 17: 251-256 (2003).

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*

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In addition to the mosquitocidal 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 mosquitocidal 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 (LC_{50}, 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 mosquitocidal 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

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It was initially demonstrated that helices 4 and 5 of the Cry4Ba mosquito-larvicidal protein from *Bacillus thuringiensis* subsp. *israelensis* (Bti) are toxic determinants 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 (POPC/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 PAPERS. Thursday, 4:00-5:45

MICROBIAL CONTROL 3

Contributed paper. Thursday, 4:00. 219

Host plant determines efficacy of *Beauveria bassiana* against western flower thrips

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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 Dubois[1], Clifford S. Gold[1], Pamela Paparu[1], Juliet Akello[1], Ekwamu Adipala[2], and Daniel Coyne[1]

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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. Gold[1], Caroline Nankinga[1,2], William Tinzaara[1], Thomas Dubois[1], Juliet Akello[1], and Wilberforce Tushemereirwe[2]

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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

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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 2.5×10^{13} 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 of second-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

not statistically significant (ANOVA based on log transformed numbers of adults: $F_{[1,3]} = 0.90$, $P = 0.41$; ANOVA based on arcsine transformed % control: $F_{[1,3]} = 0.14$, $P = 0.74$). The results suggest that the initial night following inoculation of the host with this slow acting pathogen is not a critical factor in determining efficacy. Inoculation of conidia onto protected areas of the host cuticle and multiple nights of favorable conditions or extended periods of wet weather conditions may be more important factors.

Contributed paper. Thursday, 5:00. 223

Use of genetic diversity in *Beauveria bassiana* for improving the biological control of the coffee berry borer

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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 $1x10^6$ spores/ml of each, 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, 5:15. 224

The application of *Metarhizium anisopliae* and *Beauveria bassiana* for the control of the longicorn beetle borer *Agrianome spinicolis* (Cerambycidae) in pecan trees

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Agrianome spinicolis (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.

Contributed paper. Thursday, 5:30. 225

Microbial control of the spotted stemborer *Chilo partellus* with *Beauveria bassiana* and *Metarhizium anisopliae* from Ethiopia and South Africa

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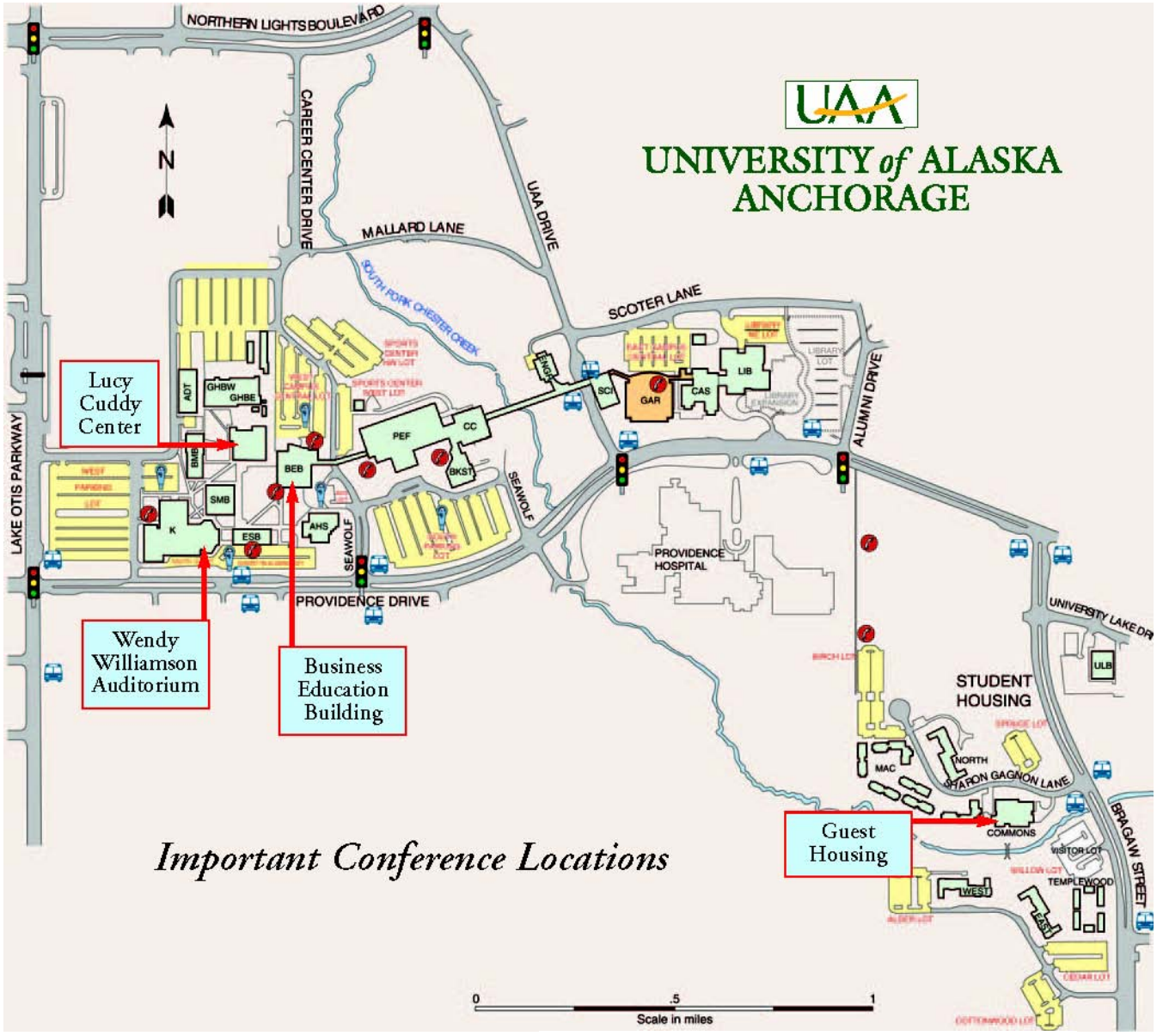
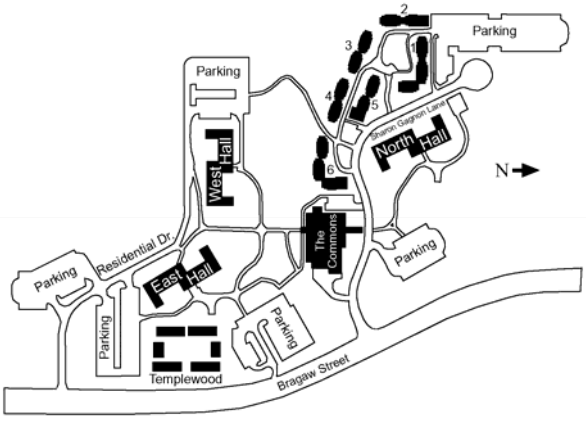
Microbial control studies were conducted with isolates of entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* from Ethiopia and South Africa againsst the spotted stem borer *Chilo partellus*. Four isolates of *B. bassiana* and six isolates of *M. anisopliae* were tested against second instar larva. Of these isolates, *B. bassiana* (BB-01) and *M. anisopliae* (PPRC-4, PPPRC-19, PPRC-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 30ºC 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 trails, conidial suspension of 2 x E8 condia/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.

AUTHOR INDEX
Abstract no. 193 indicates oral presentation; B-12 indicates poster presentation

Abstract no. 114 indicates oral presentation; B-34 indicates poster presentation



GUEST HOUSING MAP►



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.