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STU indicates papers being judged for graduate student presentation awards
SATURDAY - 26 July

8:30–5:00 SIP Council Meeting Lake Champlain
1:00–5:00 Registration Mezzanine
7:00–9:00 Mixer Adirondack Ballroom

SUNDAY - 27 July

7:30–9:00 Registration Mezzanine

Opening Ceremonies and SIP Founders’ Memorial Lecture

Sunday, 8:30-10:00. Adirondack Ballroom

Opening Ceremonies
John Burand, Chair, Organizing Committee
Harry Kay, President, SIP

Founder’s Memorial Lecture
Dudley Pinnock, Chair, Founders’ Lecture Committee
Honnee: LOIS MILLER
Lecturer: ROBERT R. GRANADOS

10:00–10:30 BREAK Green Mt. Atrium

Plenary Symposium
Sunday, 10:30–12:30. Green Mt. Ballroom

Pathogen-midgut interactions
Organizers/Moderators: Loy Volkman, Sarjeet Gill.

10:30 Mosquito midgut as a physical and biological barrier to malaria transmission. M Shahabuddin. Lab. of Malaria and Vector Res., National Inst. of Allergy and Infectious Diseases, National Inst. of Health, Bethesda, MD, USA.

11:00 Arbovirus-vector interactions in midguts of the mosquito, Aedes aegypti. KE Olson, KE Bennett, I Sanchez-Vargas, C Barillas-Mury, CD Blair, W.C. Black IV, BJ Beaty. Arthropod-borne and Infectious Diseases Lab., Foothills Research Campus, Dept. of Microbiology, Immunology, and Pathology, Colorado State Univ., Fort Collins, CO, USA.

11:30 The complex relationship between a simple RNA virus and its heliothine insect host. T Hanzlik, K Gordon. CSIRO Entomol., Canberra, ACT Australia.

12:00 Midgut barriers to baculovirus infection. JO Washburn, LE Volkman. Dept. of Plant and Microbial Biology, Univ. of California, Berkeley, CA, USA.

12:30–2:00 LUNCH Adirondack Ballroom

Post-Plenary Symposium
Sunday, 2:00-4:00. Green Mt. Ballrm-A

New approaches for studying toxicity, infection and pathogenesis
Organizers: Loy E. Volkman, Sarjeet Gill.

2:00 The role of RNA interference in arbovirus infections of mosquitoes, CD Blair, EE Travanty, I Sanchez-Vargas, KM Keene, KE Olson, BJ Beaty. Arthropod-borne and Infectious Diseases Lab., Dept. of Microbiol., Immunol. and Pathol., Colorado State Univ., Fort Collins, CO, USA.


2:48 Beet armyworm midgut gene expression correlated with sensitivity or resistance to Bacillus thuringiensis delta-endotoxin Cry1Ca. RA de Maagd1, PL Bakker1, T Gechev1, T-Y Man1, S Herrero2, WJ Moar3, Plant Res. International, Wageningen, The Netherlands; 1Dept. of Entomology, Auburn Univ., Auburn, AL, USA.

3:12 Proteomic analyses of Bacillus thuringiensis toxin – insect midgut interactions. MJ Adam1,2, RJ McNall1. Biochem. & Molecular Biology and Entomology, Univ. of Georgia, Athens, GA, USA.

3:36 Tracking the infection process of Bacillus thuringiensis in the insect. C Nielsen-LeRoux1,2, C Buisson1, P Nel1, M Haji1, S Fedhila1, E Guillemer1, L Fiette1, D Lereclus1,2, Unité Génétique Microb. et Environ., INRA, la Minière, Gouyancourt, France; 1Unité de Biochim. Microb. et 2Unité d’Histotechnol. et Pathologie, Inst. Pasteur, Paris, France.

SUNDAY - 27 July

Symposium (Div. of Fungi) Sunday, 2:00-4:00. Green Mt. Ballrm–C

Conservation microbial biocontrol
Organizer/Moderator: Paresh Shah.

2:00 Conservation of Neozygites fresenii in cotton. D Steinkraus, Dept. of Entomology, Univ. of Arkansas, Fayetteville, AR, USA.

2:30 Managed field margins as refugia for Pandora neaphidica. PA Shah, JK Pell. Plant and Invertebrate Ecology Div., Rothamsted Research, Harpenden, Herts., UK.


4:00 Conservation of natural enemies of weeds and plant pathogens. HC Evans. CABI Bioscience, UK Centre (Ascot), Silwood Park, Ascot, Berks., UK.

Contributed Papers Sunday, 2:00–4:00. Lake Champlain

MICROSPORIDIA
Moderator: Leellen Solter.

2:00 Protein fingerprinting microsporidian isolates from European populations of Lymantria dispar. LF Solter1, PF Solter2, DK Pilarska2, ML McManus1. 1Illinois Natural History Survey, Urbana, IL, USA; 2Univ. of Illinois, Veterinary Pathobiology, Urbana, IL, USA; 3Bulgarian Acad. of Sciences, Inst. of Zoology, Sofia, Bulgaria; 4USDA Forest Service, Hamden, CT, USA.

2:15 Is permisiveness of Lymantria dispar larvae to microsporidian infections determined by the host’s immune response? G Hosh1,2, LF Solter2, A Schopf2. 1Center for Economic Entomology, Illinois Natural History Survey, Champaign, IL, USA; 2Inst. of Forest Entomol., BOKU-Univ. of Nat. Res. and Appl. Life Sciences, Vienna, Austria.

2:30 Factors affecting transmission of the microsporidian, Nosema fumiferanae, a natural pathogen of the spruce budworm. C Campbell1, S Smith2, K van Frankenhuysen2. 1Faculty of Forestry, Univ. of Toronto, Toronto, ON, Canada; 2Great Lakes Forestry Centre, Canadian Forest Service, Natural Resources Canada, Sault Ste. Marie, ON, Canada.
2:45 Modelling the transmission of an insect pathogen (Microsporidia) on its host, Lymantria dispar L. – a forest pest insect. D Goetz1, D Onstad2, D Crowder2, A Linde1. 1Fachhochschule Eberswalde, Dept. of Forestry, Appl. Ecology, Alfred-Moller-Str. 1, 16225 Eberswalde, Germany; 2Plant Sciences Lab., MC-634, Univ. of Illinois, Urbana IL, USA.


4:00–4:20 BREAK Green Mt. atrium

Symposium (Div. of Microsporidia) Sunday, 4:20-6:20, Lk. Champlain
Evolutionary strategies and adaptations for survival among microsporidian parasites in aquatic ecosystems
Organizer/Moderator: Theodore Andreadis.

4:20 Vertical transmission and sex ratio distortion in the Microsporidia. AM Dunn, School of Biology, Univ. of Leeds, Leeds, UK.


5:20 Population and community level effects of microsporidia in trout stream food webs. SL Kohler1, MJ Wiley1. 1Envr. Studies Program and Dept. Biol. Sciences, Western Michigan Univ., Kalamazoo, MI, USA; 2School of Nat. Res. and Environ., Univ. of Michigan, Ann Arbor, MI, USA.

5:50 Evolutionary strategies and adaptations for survival among mosquito-parasitic microsporidia and their intermediate copepod hosts. TG Andreadis. The Connecticut Agric. Expt. Station, New Haven, CT, USA.

4:20 Isolation and characterization of baculoviruses from greenhouse populations of Trichoplusia ni. M Erlandson1, S Newhouse1, A Janaat2, K Moore1, J Myers2, D Thelmann1. 1Agric. and Agri-Food Canada, Saskatoon Research Centre, Saskatoon, SK, Canada; 2Dept. of Zoology, Univ. of British Columbia, Vancouver, BC, Canada; 3Agriculture and Agri-Food Canada, Pacific Agri-Food Research Centre, Summerland, BC, Canada.


4:50 Field and safety assessment of genetically modified Helicoverpa armigera nucleopolyhedrovirus as a commercial insecticide. X Sun1,2, H Wang1, X Sun3, X Chen1, W van der Werf1, JM Vlak1, Z Hu4. 1Joint-Lab. of Invertebr. Virol. & Key Lab. of Molec. Virol., Wuhan Inst. of Virol., Chinese Acad. of Sci., Wuhan, China; 2Crop and Weed Ecology Group and 4Lab. of Virology, Wageningen Univ., The Netherlands.

5:05 Sunshine and infection by NPV in field populations of western tent caterpillars. JH Myers, L Frid. Dept. of Zoology and Faculty of Agricultural Sciences, Univ. of British Columbia, Vancouver, BC, Canada.


5:35 Advances towards improving the insecticidal properties of AgMNPV. V Romanowski1,2, El Arana1, CB McGrath1, M Eire1, A Sciocco-Cap1, AV Goldberg2, P Ghiurighelli2, JF Pinedo3, F Moscardi4, BM Ribeiro5, 1IBBBM, Fac. Ciencias Exactas, Univ. Nacional de La Plata; 2IMYZA, INTA Castelar, 3Univ. Nacional de Quilmes; Argentina; 4Dept. of Biologia Celular, Univ. de Brasilia; 5CNPSo-EMBRAPA, Londrina, Brazil.

5:50 Comparing transmission between LdNPV strains: “liquefying” vs. “nonliquefying.” V D’Amico1, J Podgawite1, K Webb2, K Thorpe3, R Fuester2, M Valenti1, R Pfeiffer1, T Taylor1, J Slavicek1. 1USDA For. Serv., Hamden, CT, USA; 2USDA, ARS, Beltsville, MD, USA; 3USDA, ARS, Newark, DE, USA; 4Delaware Dept. Agric., Dover, DE, USA; 5Delaware State Univ., Dover, DE, USA; 6USDA For. Service, Delaware, OH, USA.


BACTERIA – 1
Moderator: Brian Federici.

4:20 Inheritance of resistance to Bacillus thuringiensis kurstaki in Trichoplusia ni. AF Janaat1, J Myers1,2. 1Dept. of Zoool., Univ. of British Columbia, Vancouver, Canada.

4:35 Understanding and overcoming resistance of Plutella xylostella to Bacillus thuringiensis Cry1Ac toxin. R Gatsi1, M Kouskoura1, A Sayyed1, D Wright2. 1School of Biol. Sci., Univ. of Sussex, UK; 2Dept. of Biol. Sci., Imperial College, UK.

4:50 Resistance to Bacillus thuringiensis endotoxins in the European corn borer (Lepidoptera: Crambidae). H Li1, J Gonzalez-Caberra1, B Oppert1, J Ferrie2, RA Higgins3, LL Buschman1, KY Zhu1, F Huang1. 1Dept. of Entomol., Kansas State Univ., Manhattan, KS, USA; 2Dept. of Genetics, Univ. of Valencia, Burjassot (Valencia), Spain; 3Grain Marketing and Production Res. Center, USDA ARS, Manhattan, KS, USA.

5:05 The effect of genetically modified insect-resistant Brassica plants on non-target invertebrates. RE Collier1, RH Collier1, CC Payne1. 1Horticul. Res. Internat., Wellesbourne, Warwick, UK; 2The Univ. of Reading, Whiteknights, Reading, UK.

5:20 Studying Cry1C-resistance mechanisms by using Sf9 cells. D Avisar1, B Sneh1, N Chejanovsky1, A Zilberstein1. Dept.of Plant Science, Tel Aviv Univ., Tel Aviv, Israel; 2Dept. of Entomology, Plant Protection Institute, Volcani Center, Bet Dagan, Israel.

5:35 Selection with Bacillus sphaericus Plus Cyl1AA from Bacillus thuringiensis subsp. israeilensis: effect on Bacillus sphaericus resistance in mosquitoes. MC Wirth1, J A Jannino1, BA Federici2, WE Walton2. 1Dept. of Entomology & Interdepartmental Graduate Program in Genetics, Univ. of California, Riverside, CA, USA.

Contributed Papers Sunday, 4:20-6:20. Green Mountain–A

VIRUSES – 1
Moderator: Bryony Bonning.

4:20 Modelling the transmission of an insect pathogen Helicoverpa armigera nucleopolyhedrovirus as a commercial insecticide. X Sun1,2, H Wang1, X Sun3, X Chen1, W van der Werf1, JM Vlak1, Z Hu4. 1Joint-Lab. of Invertebr. Virol. & Key Lab. of Molec. Virol., Wuhan Inst. of Virol., Chinese Acad. of Sci., Wuhan, China; 2Crop and Weed Ecology Group and 4Lab. of Virology, Wageningen Univ., The Netherlands.

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5:50 Comparing transmission between LdNPV strains: “liquefying” vs. “nonliquefying.” V D’Amico1, J Podgawite1, K Webb2, K Thorpe3, R Fuester2, M Valenti1, R Pfeiffer1, T Taylor1, J Slavicek1. 1USDA For. Serv., Hamden, CT, USA; 2USDA, ARS, Beltsville, MD, USA; 3USDA, ARS, Newark, DE, USA; 4Delaware Dept. Agric., Dover, DE, USA; 5Delaware State Univ., Dover, DE, USA; 6USDA For. Service, Delaware, OH, USA.
Phylogenetic diversity within *Bacillus thuringiensis* and *Bacillus cereus* isolates: Only one group has pathogenic or toxigenic properties in vertebrates. PJ Jackson, KK Hill, LO Ticknor, CH Helma, RT Okinaka. Bioscience Div., Los Alamos National Lab., Los Alamos, NM, USA.

**Fungi – 1**

*Isolate selection and formulation of Beauveria bassiana for controlling tarnished plant bug, *Lygus lineolaris* (Heteroptera: Miridae) in wild host plants, JE Leland1, RW Behie2. 1USDA-ARS, Southern Insect Management Res. Unit, Stoneville, MS, USA; 2USDA-ARS, National Center for Agric. Utiliz. Res., Peoria, IL, USA.*

*Impact of Beauveria bassiana on Western tarnished plant bug, MR McGuire. USDA-ARS, Shafter, CA, USA.*

*Evaluation of bee pollinators as vectors of Beauveria bassiana for control of the tarnished plant bug and western flower thrips on greenhouse peppers. MS Alamzara1, JL Ship2, AB Broadbent3, PG Kevan4. 1Univ. of Guelph, ON, Canada; 2Agric. and Agri-Food Canada, Harrow, ON, Canada; 3Agric. and Agri-Food Canada, London, ON, Canada.*

*The effect of changing application rate, volume, and interval on acquisition of Beauveria bassiana conidia by western flower thrips and resulting control in garden impatiens, TA Upping1, SP Wright2, JP Sanderson3. 1Cornell Univ., Ithaca, NY; 2USDA-ARS Ithaca, NY, USA.*


*Management of sucking pests with Beauveria bassiana in Australia, K Knight, D Holdom, C Hauxwell. QDPI Biopesticides Unit, Agency for Food and Fibre Sciences, Indooroopilly, Queensland, Australia.*


*Comparative virulence and host specificity of Beauveria bassiana isolates assayed against lepidopteran pests of vegetable crops, SP Wright1, ME Ramos1, JE Williams1, PB Avery1, ST Jaronski1, JD Vandenberg1. 1USDA-ARS, U.S. Plant, Soil, & Nutrition Lab., Tower Rd, Ithaca, NY, USA; 2Formerly Mycotech Corp, Butte, MT, USA [current address: USDA-ARS Northern Plains Agric. Res. Lab., Sidney, MT, USA]; 3Lee Academy, Lee, ME, USA.*

**SIP Division Business Meetings:** Sunday evening

**Bacteria**

(7:00-8:00p) Lake Champlain

**Viruses**

(7:00-8:00p) Adirondack Ballrm

**Microbial Control**

(8:00-9:00p) Green Mt. Ballrm

Workshop (approx. 9:00): Microbial control products: What’s in the pipeline? (J. Lord, Organizer)

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**DINNER**

6:20–8:00 Pizza Party! (no host / tickets required)

**MONDAY - 28 July**

**Sympos. (Div. of Microb. Control) Monday, 8:00-10:00. Green Mt.–A**

**Is bigger always better? A comparison of industrial-scale vs. cottage industry-scale production of microbial pesticides**

Organizers/Moderators: Wendy Gelernter, Lawrence A. Lacey.

8:00 Introduction, W Gelernter. Pace Consulting, San Diego, CA, USA

8:05 Do we have it in the bag? - Production of *Metarhizium anisopliae*. JA Langewald1, NE Jenkins2, B Ali3, M Brüntrup1, D Moore1. 1IITA, Cotonou, Benin; 2CABI Bioscience, Silwood Park, Ascot, UK; 3CABI Bioscience, Caribbean and Latin America Centre, Trinidad & Tobago; 4Freelance consultant, Stuttgart, Germany.

8:28 Entomopathogenic nematode production. DI Shapiro-Ilan. USDA-ARS, SE Fruit & Tree Nut Res. Lab., Byron, GA, USA.

8:51 Production of biopesticides in developing countries: the roles of cottage industry, NGOs, state sector enterprises and private commercial producers in Asia. D Grzywacz1, U Ketunuti2, H Warburton1. 1Natural Resources Inst., Univ. of Greenwich, Chatham Maritime, Kent, UK; 2Dept. of Agric., Chaturachak, Bangkok, Thailand.

9:14 Commercializing mycoinsecticides: The U.S. experience. ST Jaronski. USDA REE ARS NPRL, Sidney MT USA; formerly Manager, Biopesticide R&D, Mycotech Corp., Butte, MT, USA.

9:37 “Evolutionary ecology” of the microbial pesticide industry: Does size really matter? MB Dimock. Certis USA, LLC., Columbia, MD, USA.

**Symposium (Division of Viruses) Monday, 8:00-10:00. Green Mt.–C**

**Insect resistance mechanisms to viruses: Beyond the midgut**

Organizers: Kelli Hoover, Diana Cox-Foster.

Moderator: Kelli Hoover.

8:00 Clues from viral genomes to insect anti-viral immune responses, BA Webb. Dept. of Entomology, Univ. of Kentucky, Lexington, KY, USA.

8:50 Apoptosis as a defense response against virus infection in insects. TE Clarke, L Heaton, RJ Clem, Molecular, Cellular, and Developmental Biol. Program, Division of Biol., Kansas State Univ., Manhattan, KS, USA.

9:15 Virucidal activity against HSNPV in plasma of Heliothis virescens. HIR Popham, KS Shelby, SL Brandt, USDA ARS Biol. Control of Insects Res. Lab., Columbia, MO, USA

9:40 Intra-stadial developmental resistance of gypsy moth to its own baculovirus. D Cox-Foster, M Grove, S Su, J McNeil, K Hoover, Dept. of Entomology, The Pennsylvania State Univ., University Park, PA, USA.

Contributed Papers

Mondays, 8:00-10:00. Lake Champlain

BACTERIA – 2

Moderator: Leah Bauer.

8:00 Enduring toxicity of transgenic Anabaena expressing mosquito larvicidal genes from Bacillus thuringiensis subsp. israelensis. B Manasherob,1,2 ZN Oteno-Ayayo,1,4 E Ben-Dov,1,4 R Maskovsky,2,4 S Boussiba,3,4 A Zaritsky1,4 1Dept. of Life Sciences and 2Microalgal Biotechnol. Lab., Ben-Gurion Univ. of the Negev, Beer-Sheva, Israel; 3BioSan Ltd., Ariel, Israel; 4Dept. of Math., Environ. & Natural Sci., Solusi Univ., Bulawayo, Zimbabwe.

8:15 Diamondback moth vs. Bt-B. napus/Bt-B. rapa: Who will win? L Beau7, SL Warick2, P Mason2, B Zhu3, CN Stewart Jr., 1Agric. and Agri-Food Canada, Saskatchewan, Canada; 2Agric. and Agri-Food Canada, Ottawa, Ontario, Canada; 3Environ. Canada, National Water Res. Inst., Saskatoon, Saskatchewan, Canada; 4Dept. of Plant Sciences and Landscape Systems, Univ. of Tennessee, Knoxville, TN, USA.

8:30 Emerald ash borer susceptibility to Bacillus thuringiensis var. kurstaki EG7673. LS Bauer1,2, DL Miller1, USDA Forest Service, North Central Res. Station, East Lansing, MI, USA; 2Dept. of Entomology, Michigan State Univ., East Lansing, MI, USA.

8:45 Diversity of bacteria associated with the gut of stem boring beetles (Coleoptera: Cerambycidae, Scolytidae). I Delalibera Jr.,1 J Handelsman,2 K Raffa,1 1Dept. of Entomology, 2Dept. of Plant Pathology, Univ. of Wisconsin, Madison WI 53706, USA.

9:00 Preliminary observations on effects of Bt-corn on non-target soil Collembola. M Brownbridge, Entomol. Res. Lab., Univ. of Vermont, Burlington, VT, USA.

9:15 Comparative analysis of efficacy of different strains of Bacillus thuringiensis subsp. thuringiensis against Tortrix viridana (Lepidoptera, Tortricidae) in field conditions. AV Ivanov1, AP Simchuk1, IG Peletskaya1, SY Gouli2, 1Dept. of Ecology, V.I. Vernadsky National Univ., Simferopol, Ukraine; 2V.I. Vernadsky National Univ., Simferopol, Crimea, Ukraine.

9:30 Genomic response of C. elegans to Bt crystal protein intoxication. D Huffman, BV Aronson. Sect. of Cellular and Developmental Biology, Univ. of California-San Diego, La Jolla, CA, USA.

10:00–10:30 BREAK

SYMPOSIUM (Cross-Div.) Monday, 10:30–12:30. Green Mt.–A

Diseases and pathobiology of aquatic invertebrates

Organizer/Moderator: Robert Anderson.

10:30 Quahog Parasite Unknown, an important disease of the hard clam, Mercenaria mercenaria. R Smolowiz. Marine Biological Laboratory, Woods Hole, MA, USA.

10:50 Molecular diagnostics and phylogenetic analysis of Quahog Parasite Unknown (QPX). NA Stokes1, LM Ragone Calvo1, KS Reece2, EM Burreson1, 1Dept. of Envir. & Aquatic Animal Health and 2Aquaculture Genetics and Breeding Technology Center, Virginia Institute of Marine Science, Gloucester Point, VA, USA.

11:10 The first occurrence of MSX disease in Canada – aberrant pathology and discovery of SSO. SE McGладdery, MF Stephenson, N Gagné, A Locke. Fisheries and Oceans Canada, Gulf Fisheries Centre, Moncton, New Brunswick, Canada.

11:30 Development of biochemical indicators of stress for bivalves: recent studies on heat shock proteins and proteases. N Ross1, E Egosimbiba1, N Bru1, M Bricelj1, T MacRae2, J Harding1, J Coutrier1, J Parsons2. 1National Res. Council, Inst. for Marine Biosciences, Halifax, NS, Canada; 2Dept. of Biology, Dalhousie Univ., Halifax, NS, Canada; 3Fisheries and Marine Inst. of Memorial Univ. of Newfoundland, St. John's, NF, Canada.

11:50 Fixed phagocytes of the digestive gland - A mostly ignored part of the immune system of lobsters (and other crustaceans). JR Factor. Div. of Nat. Sciences, Purchase College, State Univ. of New York, Purchase, NY, USA.


Contributed Papers

Mondays, 10:30-12:15. Lake Champlain

NEMATODES

Moderator: Albrecht Koppenhöfer.

10:30 Genomic fingerprinting of Xenorhabdus spp. using repetitive sequences and PCR. HI Smith1, BJ Adams1, JB Jones1, FJ Louws1. 1Dept. of Entomology and Nematology, 2Dept. of Plant Pathol., Univ. of Florida, Gainesville, FL, USA; 3Dept. of Plant Pathol., North Carolina State Univ., Raleigh, NC, USA.

10:45 Evaluation of entomopathogenic nematode strains for the control of Anoplophora glabripennis. D Fallon1, L Solter1, M Keena2, J Cate3, M McManus2, L Hanks3. 1Illinois Natural History Survey, Univ. of Illinois, NSRC, Urbana, IL, USA; 2USDA Foresty Service, Northeastern Research Station, Hamden, CT, USA; 3Integrated Biocontrol Systems, Inc., Aurora, IN, USA; 4Entomology Dep., Univ. of Illinois, Urbana, IL, USA.

11:00 Susceptibility of the European crane fly to four entomopathogenic nematodes (Steinernematidae and Heterorhabditidae). L Simard1, G Belair2, J Dionne1. 1Centre de Recherche en Horticulture, Univ. Laval, Québec, Canada; 2Agric. and Agri-Food Canada, St-Jean-sur-Richelieu, Quebec, Canada.

11:30 The effect of inundative application of entomopathogenic nematodes on soil processes: A microcosm study. EAB De Nardo, 1 2 PS Grewal, 1 D McCartney, 1 BR Stinner. 1 Dept. of Entomology, Ohio State Univ., Ohio Agr. Research and Devel. Center, OARDC, Wooster, OH, USA; 2 Permanent Addr.: Embrapa Meio Ambiente, Brazil.

11:45 Differential susceptibility of larval instars of the citrus root weevil, Diaprepes abbreviatus, to the entomopathogenic nematode, Steirnema riobrave. RJ Stuart, CW McCoy. Univ. of Florida, CREC-IFAS, Lake Alfred, FL, USA.

12:00 Effect of insect food plant and selection on infectivity, sex ratio, and melanization of Steirnema spp. in Diabrotica undecimpunctata howardi. ME Barbereck, 1 J Wang, 2 C Browne. 1 Dept. of Entomology and 2 Dept. of Statistics, North Carolina State Univ., Raleigh, NC 27695, USA; *current addr.: Dept. of Entomol., The Pennsylvania State Univ., University Park, PA16802, USA.

**Contributed Papers**

**Monday, 10:30-12:30. Green Mt.–C**

**MICROBIAL CONTROL**

Moderator: Michael Brownbridge.

10:30 Exploitation of natural enemies and pathogens to activate a persistent baculovirus in field and laboratory populations of the cabbage moth Mamestra brassicae. C Nixon, 1 R Possee, 2 R Hails, 1 L King. 1 Oxford Brookes Univ., Oxford, UK; 2 NERC Centre for Ecology and Hydrology, Oxford, UK.

10:45 Improvements in the large scale production of the velvetbean caterpillar, Anticarsia gemmatalis, nucleopolyhedrovirus in the laboratory. B Santos, 1 F Moscardi. 1 Dept. of Agronomy, Univ. Federal do Para, Curitiba, PR, Brazil; 2 Embrapa Soja, Londrina, PR, Brazil.

11:00 Trends of mass production of microbial pesticides in Russia. MV Shternshtein, 1 VV Gouli. 1 Novosibirsk State Agrarian Univ., Novosibirsk, Russia; 2 Univ. of Vermont, Burlington, VT, USA.

11:15 Can composted mulches create an environment that promotes the incidence and activity of natural enemies for control of avocado thrips in Californian avocado orchards? M Brownbridge, 1 P. De Ley, 1 I.T. De Ley, 2 and M. Hoddle. 1 Entomology Res. Lab., Univ. of Vermont, Burlington, VT, USA; 2 Dept. Nematol. and 3 Dept. Entomol., Univ. of California, Riverside, CA, USA.

11:30 Non-infectious disease: A neglected paradigm? SD Costa. Entomology Research Lab., Dept. of Plant and Soil Science, Burlington, VT, USA.

1:45 The definitions and measurement of pathogenicity and virulence. S Thomas, J Elkinton. Dept. of Entomology, Univ. of Massachusetts, Amherst, MA, USA.

12:00 Possibility for enhancement of practical pest control based on *Hyphomycetes fungi*, VV Gouli, SY Gouli. Entomol. Res. Lab., Univ. Vermont, Burlington, VT, USA.
F-12  Complementation techniques and parameters used in compatibility tests between Beauveria bassiana and chemical pesticides in vitro.  PMJU Neves, RZ Silva.  Depto. Agronom., Univ. Estadual de Londrina, PR, Brazil.

F-13  Effect of growing media and water volume on conidial production of Beauveria bassiana and Metarhizium anisopliae.  M El Hamidi, M Skinner, BL Parker, V Gosli, S Gouli.  Entomol. Res. Laboratory, Univ. of Vermont, Burlington, VT, USA.


F-17  Development of a biologically based pest and disease management system in sugar beets.  ST Jaronski, J Grace, S Gaffri.  USDA, ARS Northern Plains Agricultural Lab, Sidney MT, USA.


F-19  Detection of strains of Metarhizium within infected sugar cane borer, Diatraea saccharalis, using specific primers.  RHR Destéfano, SAL Destéfano, CT Messias.  1State Univ. of Campinas, Campinas, SP, Brazil; 2Inst. Biológico, Campinas, SP, Brazil.

F-20  Variability in response to heat among strains of Metarhizium anisopliae isolated from sites at latitudes from 61°N to 54°S.  DEN Rangel, GUL Braga, AJ Anderson, DW Roberts.  1Dept. of Biology, Utah State Univ., Logan, Utah, USA.


F-22  Efficacy of locally collected isolates of Metarhizium anisopliae var acridum and Metarhizium flavoviride on three acidid pests in Senegal, West Africa.  A Niasay, K Badji, L Vaughan.  1Direction de la Protection des Végétaux, Dakar, Senegal; 2Office of International Research, Education, and Development, Virginia Polytechnic Inst. and State Univ., Blacksburg, VA, USA.

F-23  Influence of submerged cultivation additives and formulation ingredients on the tolerance of blastospores of Metarhizium anisopliae var. acridum to thermic stress under fluctuating regimes.  J Paraguas, N Smits, C Vidal, W Meikic, G Mer, N Issaly, L Vaughan.  1UMR, Centre de Biologie et de Gestion des Populations, INRA, Montpellier, France; 2European Biol. Control Lab., USDA-ARS, Montferrier, France; 3UMR, Microbiol., INRA, Dijon, France; 4Office of International Research and Development, Virginia Tech, Blacksburg, VA, USA.


F-26  Epizootic potential of Trinidadian strains of Paecilomyces fumosoroseus against Trialeurodes vaporariorum under laboratory conditions.  PB Avery, J Faul, M Simmonds.  1School of Biol. and Chem. Sciences, Birkbeck College, London, UK; 2Lee Academy, Lee, ME, USA; 3Royal Botanic Gardens, Kew, Richmond, Surrey, UK.

F-27  Individual and combined effects of Paecilomyces fumosoroseus and Encarsia formosa for control of Trialeurodes vaporariorum on beans and Regal geraniums.  PB Avery, J Faul, M Simmonds.  1School of Biological and Chemical Sciences, Birkbeck College, London, UK; 2Lee Academy, Lee, ME, USA; 3Royal Botanic Gardens, Kew, Richmond, Surrey, UK.


F-29  Phylogenetic relationships of entomopathogenic fungi based on mitochondrial SSU rDNA sequences.  DR Sosa-Gomez, KT Hodge, RA Hunter, PB Avery, 2J Faull, M Simmonds.  1School of Biological and Chemical Sciences, Birkbeck College, London, UK; 2Lee Academy, Lee, ME, USA; 3Royal Botanic Gardens, Kew, Richmond, Surrey, UK.

F-30  Field incidence of Nomuraea rileyi and evidence that multiple strains are present in the same field.  D Kalkar, GR Carner, Y Kusumah.  Dept of Entomol., Clemson Univ., Clemson, SC, USA.

F-31  Inhibition of the host immune reaction by entomopathogenic fungus Nomuraea rileyi.  H Hiromori, D Yaginuma, M Hatsukade.  Lab. of Applied Entomology, Fac. of Agriculture, Shizouka Univ., Shizuoka, Japan.


F-33  Mycopathogens of Homalodisca coagulata, the Glassy-
STU  Winged Sharpshooter. TM Conklin, D Purcell, RF Mizell, DG Boucias. Dept. of Entomol. & Nematol., Univ. of Florida, Gainesville, USA.

F-34  Influence of the entomopathogenic fungus, Verticillium lecanii, on an aphid parasitoid, Aphidius colemani, and a predator, Chrysopa pallens. JJ Kim1, DJ Im1, KC Kim1, DR Choi1, DW Roberts2. ‘Div. of Entomol., NIAST, RDA, Korea; 1Dept. of Agrobiol., Chonnam National Univ., Korea; 1Dept. Biology, Utah State Univ., USA.

F-35  Comparison of Japanese and American isolates of Entomophaga maimai. S Thomas, J Elkinton. Dept. of Entomol. and Nematol., Univ. of Massachusetts, Amherst, MA, USA.


F-37  Do Vicia faba plants use the aphid pathogen Pandora neaphidids as a bodyguard? J Baverstock1,2, PG Alderson2, SL Elliott1, JK Pelt1.1 Plant and Invertebrate Ecology Division, Rothamsted Research, UK; 2Division of Agricultural Sciences, The Univ. of Nottingham, UK; NERC Centre for Population Biology, Imperial College, Silwood Park, UK.

F-38  In vitro interactions between two fungal pathogens of Plutella xylostella: Pandora bluntschi and Zoophthora radicans. A Guzman Franco1,2, PG Alderson1, JK Pelt1.1 Plant and Invertebr. Ecology Division, Rothamsted Research, Harpenden, UK; 2Division of Agricultural Sciences, Univ. of Nottingham, UK.


F-40  In vitro development of Helicosporidium. M Borns1.1 S Shapiro, J Becnel, D Boucias. Dept. of Entomology and Nematology, Univ. of Florida, Gainesville, FL, USA.


MP-4  Trypanosomatid infections affect male Aquarius remigis body size: Implications for gerrid mating interactions. KC Gurski, MA Ebbert. Dept. of Zoology, Miami Univ., Oxford, OH, USA.

MP-5  An undescribed microsporidium from Lygus hesperus and Lygus lineolaris. DA Street1, E Villavaso2.1 USDA-ARS, Biol. Control and Mass Rearing Res. Unit, Mississippi State, MS, USA; 2NERC Centre for Population Biology, Imperial College, Harpenden, UK.

STU  In vitro development of Helicosporidium. M Borns1.1 S Shapiro, J Becnel, D Boucias. Dept. of Entomology and Nematology, Univ. of Florida, Gainesville, FL, USA.

STU  Microsporidia & Protozoa

MC-1  The USDA-ARS National Biological Control Laboratory: Expectations for the new facility. DA Streett. USDA-ARS-Biological Control and Mass Rearing Research Unit, Mississippi State, MS, USA.

MC-2  Nematodes and entomopathogenic fungi associated with termites. WG Meikle1, G Mercader1, AA Kirk1, M-C Hou1, L Sawicki2, E Derouault1, A Peppuy1, Y He1, A Reid1, PC Quimbay3.1 European Biological Control Lab, USDA - ARS, Campus International de Baillarguet, Montferrier sur Lez, St. Gely du Fesc, France; 2Observatoire Régional de Lutte Anti-Termes (ORLAT), St. Andre, La Réunion; 3Lab. of Insect Ecol., South China Agric. Univ., Wushan, Guangzhou, China; 4Edinburgh, Scotland, UK.

MC-3  Survey for natural enemies of the alfalfa snout beetle Otiorhynchus lugustici (L.) in Hungary and in New York State: Nosema otiorhynchi, entomopathogenic nematodes and entomopathogenic fungi. G Neumann, E Shilde1, A Hajek. Dept. of Entomol., Cornell Univ., Ithaca, NY, USA.

MC-4  Heritability and plasticity of immune function in the Egyptian cotton leafworm, Scutter2, K Wilson1.1 Institute of Biological Sciences, Univ. of Stirling, Stirling, UK; NERC Centre for Population Biology, Imperial College, Harpenden, UK; 2CSIRO Entomology, Wembley, WA, Australia.

MC-5  Evidence for suppression of immunity in honey bees by parasitic Varroa mites. X Yang, DL Cox-Foster. Dept. of Entomology, The Pennsylvania State Univ., University Park, PA, USA.

MC-6  Microbial control of the Colorado potato beetle in irrigated desert: combinations and alternations of Bacillus thuringiensis and Beauveria bassiana. LA Lacey, DR Horton. USDA-ARS, Yakima Agricultural Research Lab., Wapato, WA, USA.

MC-7  Assessing environmental risks of biological control agents: a general framework. HMT Hokkanen. Lab. of Applied Zoology, Univ. of Helsinki, Finland.

STU  Winged Sharpshooter. TM Conklin, D Purcell, RF Mizell, DG Boucias. Dept. of Entomol. & Nematol., Univ. of Florida, Gainesville, USA.

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STU  Winged Sharpshooter. TM Conklin, D Purcell, RF Mizell, DG Boucias. Dept. of Entomol. & Nematol., Univ. of Florida, Gainesville, USA.
Evasive behavior of white grub species against entomopathogenic nematodes. C Yoder, PS Grewal. Dept. of Entomol., Ohio State Univ., OARDAC, Wooster, OH, USA.

New strains of the entomopathogenic nematode, Steinernema riobravense: are they better for biological control of the citrus root weevil, Diaprepes abbreviatus? RJ Stuart, D Shapiro-Ilan, CW McCoy, Univ. of Florida, CREC-IFAS, Lake Alfred, FL, USA; USDA-ARS, Southeast Fruit and Tree Nut Res. Lab., Byron, GA, USA.

Race to death: the encapsulation response by insect hemocytes is mediated by the surface coat proteins of Heterorhabditis bacteriophora and Steinernema glaseri. DL Cox-Foster, X Li, A Kazi, E Troy, K Miller. Dept. of Entomology, Penn State Univ., University Park, PA, USA.

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**SYMPOSIUM (Cross-Div.)**

Monday, 4:30–6:40. Green Mt.-A

Host altered behavior: Host mediated or pathogen induced

Organizer/Moderator: Helen Roy

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Molecular genetic analysis and enhancement of Cry19A synthesis in Bacillus thuringiensis. JE Barboza-Corong, H-W Park, BA Federici, Dept. of Entomol. and Graduate Programs in Genetics and Microbiol., Univ. of California, Riverside, CA, USA; Instituto de Ciencias Agrícolas, Univ. de Guanajuato, Irapuato, Guanajuato, México.

Cyt1A synergizes toxicity of Bs Bin by enhancing its insertion through the mosquito midgut microvillar membrane. BA Federici, MC Wirth, JJ Johnson, H-W Park, DK Bideshi, WE Walton. Dept. of Entomology and Interdepartmental Grad. Progr. in Genet. and Microbiol., Univ. of California, Riverside, CA, USA.

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Manipulation of host behavior by entomopathogenic fungi. AE Hajesk, JE Losey, C Gilbert. Dept. of Entomology, Cornell Univ., Ithaca, NY, USA.

Host manipulation by insect baculoviruses. JS Cory. Molecular Ecology and Biocontrol Group, NERC Centre for Ecology & Hydrology, Oxford, UK.

Alteration of host physiology and mating behavior resulting from virus replication. JP Burand. Depts. of Entomology and Microbiology, Univ. of Massachusetts, Amherst, MA, USA.

Manipulation of sexual reproduction by the intracellular bacteria Wolbachia. S Bordenstein. The Marine Biological Laboratory, Josephine Bay Paul Center for Compar. Molec. Biol. and Evol., Woods Hole, MA, USA.


Behavior of nematode-infected insects and of scavengers to nematode-killed insects. HK Kaya, L Luong. Dept. of Nematology, Univ. of California, Davis, CA, USA.
SIP Division Business Meetings:
The mechanism of Ha-Vp39 binding to actin and the 5:45
Transcriptional regulation of a 5:15
The immediate early 0 protein IE0 of the 4:45
STU infected lepidopteran cells.
RNA interference in uninfected and baculovirus-
5:00 Formation of budded virus at the plasma membrane 4:30
in baculovirus-infected cells involves the localisation of 4:15
STU gp64 within lipid rafts. 4:00
Origins of replication in Cydia pomonella granulovirus. 3:45
S Shapiro, J Becnel, D Boucias. Dept. of Entomology and 3:30
Nematology, Univ. of Florida, Gainesville, FL, USA.

Symposium (Cross-Div.)
You are what you eat: Multitrophy in invertebrate pathology systems
Organizers: Kelli Hoover, Gary Felton, Patricia Stock. Moderators: Kelli Hoover, Gary Felton.

Contributed Papers
Moderator: James Slavicek.


4:45 Formation of budded virus at the plasma membrane in baculovirus-infected cells involves the localisation of gp64 within lipid rafts. PJ Haines1, AL Patmanidi1, CR Hawes1, BD Possee2, LA King1. 1School of Biol. and Molec. Sci., Oxford Brookes Univ., Gipsy Lane Campus, Oxford, UK; 2NERC Inst. of Virology and Environmental Microbiology (CEH, Oxford), Oxford, UK.

5:00 RNA interference in uninfected and baculovirus-infected lepidopteran cells. T1 Zaki1,2, JE Maruniak1,2. 1Dept. of Microbiology and Cell Science and 2Dept. of Entomol. and Nematol., Univ. of Florida, Gainesville, Florida, USA; 3Agricultural Genetic Engineering Res. Inst. (AGERI) and Agricultural Research Center, Giza, Egypt.

5:15 The Lymantria dispar nucleopolyhedrovirus enhancin 1 and 2 proteins occupy distinct envelope locations and each protein shifts its location in the absence of the other protein. JM Slavicek, HJR Popham1. USDA Forest Serv., Forestry Sci. Lab., Delaware, OH, USA; USDA ARS, Biol. Control of Insects Res. Lab., Columbia, MO, USA.

5:30 The immediate early 0 protein IE0 of the Autographa californica nucleopolyhedrovirus is not essential for viral replication. L Lu, N Chejanovsky. Entomology Dept., Inst. of Plant Prot., ARQ, The Volcani Center, Bet Dagan, Israel.

5:45 Transcriptional regulation of a Chilo iridescent virus early and late gene. R Nalpacioglu1,2, Z Demirbag1, JM Vlak1, MM van Oers1. 1Lab. of Virology, Wageningen Univ., The Netherlands; 2Dept. of Biol., Fac. of Arts & Sciences, Karadeniz Technical Univ., Trabzon, Turkey.

6:00 The mechanism of Ha-Vp39 binding to actin and the influence on proliferation and assembly of progeny virions. G Ge, S Lu, Y Qi. College of Life Sciences, Wuhan Univ., Wuhan, Hubei, P.R.China.

Symposium (Div. of Nematodes)
Genomics of entomopathogenic nematode-bacterium complexes
Organizers/Moderators: Patricia Stock, Panwinder Grewal.

8:00 Introduction. P. Stock.


9:00 Plant-mediated inhibition of disease caused by baculoviruses. K Hoover, G Felton, R Plymale. Dept. of Entomology, Penn State Univ., University Park, PA, USA.

9:20 Tri- and tetratrophic level effects on entomopathogenic nematodes. AM Kapshonhöfer. Dept. of Entomology, Rutgers Univ., New Brunswick, NJ, USA.

9:40 Interactions between nematodes, insects and other microorganisms in forest ecosystems: An assortment of symbiotic associations in detrital food webs. SP Stock. Dept. of Plant Pathol., Univ. of Arizona, Tucson, AZ, USA.

DINNER
Enjoy exploring Burlington’s many possibilities!

Workshop following business meeting at approx. 9:00p:
Molecular phylogeny and the classification of the Microsporidia.
(C. Vossbrink, organizer/speaker)
**EC Martens, K Heungens, CE Cowles, EI Vivas. Dept. of Bacteriology, Univ. of Wisconsin, Madison, WI, USA.**

11:18 Novel strategies for control of chicken mites (Derma-

**10:30 Laboratory and glasshouse evaluation of entomopatho-

genic fungus against the twospotted spider mite, Tetra-

**nyssus urticae. D Chandler, G Davidson, R Jacobson.**

Horticulture Research International, Wellesbourne, Warwick, UK; 3Stockbridge Technology Centre, Stockbridge House, Selby, UK.

10:54 Challenges in using Neocydia tanajoae as a classical biological control agent for the cassava green mite in


**11:26 Evaluation of entomopathogenic fungi for control of**

Varroa destructor, an ectoparasite of the honey bee, Apis mellifera L. G Davidson, C Birchall, P Kelble, B Ball.

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**Tuesday AM**

**8:00 Integration of Metarhizium anisopliae (Deuteromycota: Hyphomycetes) and cover crops for controlling sugar-


8:30 Is PIF quantity regulated by Spodoptera littoralis nucleopolyhedrovirus (SplNPV)? S Gutiérrez, O Simon, P Caballero, M Lopez-Ferber. Lab. de Pathologie Comparee, INRA/CNRS/UMZ, St-Christol-lez-Ales, France; 2Dept. of Produccion agraria, ETSIA, Univ. Publica de Navarra, Campus de Arrosadia s/n, Pamplona, Spain.


9:30 Trypsinization of occlusion body-derived virus from three nucleopolyhedroviruses alters infectivity to insect cell lines. DF Lynn. USDA/ARS, Insect Biocontrol Lab., Henry A. Wallace Agric. Res. Center, Beltsville, MD, USA.

**10:00 Conclusions and final remarks. P Stock, P Grewal.**

**11:00-11:30 BREAK**

**11:42 Fungi for control of ticks. M Samish, G Gindin.**


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**11:18 Novel strategies for control of chicken mites (Derma-

nyssus gallinae) using autodissemination. T Steenberg.**

Danish Pest Infestation Lab., Kgs. Lyngby, Denmark.

**11:42 Fungi for control of ticks. M Samish, G Gindin.**


**12:06 Evaluation of entomopathogenic fungi for control of**

Varroa destructor, an ectoparasite of the honey bee, Apis mellifera L. G Davidson, C Birchall, P Kelble, B Ball.
V-1 A new densivirus isolated from the african cotton bollworm, Helicoverpa armigera Hbn. (Lepidoptera: Noctuidae) in Egypt. F Gédire1, M Salah1, R El-Mergawy1, M Masri1, M El-Sheikh1, A Abd-Ala1, M Bergerin2, M El-Far2, P Tijssen1. 1Centre de Virol., Inst. de Recherche pour le Développ., Fac. of Agric., Cairo Univ., Giza, Egypt; 2Lab. de Pathol. Comparée, USTL, Montpellier, 5, France; INRS-Inst. Armand-Frappier, Laval, Québec, Canada.

V-2 Allotrophic determinants of Galleria mellonella and STU Mythimna loreyi densiviruses reside on the viral capsid protein. M El-Far1,2, Y Li1, G Gédire1,3, S Abol-Ela1, P Tijssen1. INRS-Inst Armand-Frappier, Laval, Québec, Canada; Center of Virology-IRD, Faculty of Agriculture, Cairo Univ., Egypt

V-3 Gene organization and content of the Neodiprion lecontei NPV genome. HAM Lauzon1, C Lucartti2, PJ Krell1, BM Arif1. 1Canadian Forest Service, Great Lakes Forestry Centre, Sault Ste. Marie, Ontario, Canada; 2Atlantic Forestry Centre, Fredericton, New Brunswick, Canada; 3Dept. of Microbiology, Univ. of Guelph, Guelph, Ontario, Canada.


V-5 Hz-2V genome analysis. W Kim1, JP Burand2,3. 1CL Afonso, GF Kutish, Z Lu, DL Rock1. Depts. of 'Entomology and 1Microbiology, Univ. of Massachusetts, Amherst, MA, USA; 2USD, Agric. Research Service, Plum Island Animal Disease Center, Greenport, NY, USA.

V-6 Alteration of the development of reproductive tissues in Hz-2V infected Helicoverpa zea. W Tan1, JP Burand2,3. Depts. of Entomol. and 1Microbiol., Univ., of Massachusetts, Amherst, MA, USA.

V-7 Altered mating behavior and pheromone production in female Helicoverpa zea moths infected with the insect virus Hz-2V. W Tan1, JP Burand2,3, W Kim1, S Nojima1, W Roelofs1. Depts. of 'Entomology and 1Microbiology, Univ. of Massachusetts, Amherst, MA, USA; 2Dept. of Entomology, Cornell Univ., Geneva, NY, USA.


V-10 Defective baculoviruses increase the pathogenicity issues of the virus population. O Simon1,2, P Caballero1, T Williams1, M Lopez-Ferber. 1Laboratorio de Entomología Agrícola y Patología de Insectos, Dept. de Producción Agraria, Univ. Pública de Navarra, Pamplona, Spain; 2Génétique de Virus, Laboratoire de Pathologie Comparée, INRA/CNRS/Univ. de Montpellier II. St Christol les Ales, France.

V-11 Localization and sequence analysis of the Anticarsia gemmatalis nucleopolyhedrovirus 25K FP gene. ML Souza1, MEB Castro1, FR da Silva1, W Siholder2, MRS Fedrini2. Embrapa Recursos Genéticos e Biotecnologia, Brasilia, Brazil; 3Univ. of Queensland, Brisbane, Australia

V-12 Baculovirus susceptibility, improved protein production, and resistance to nutrient stress by new Trichopilia ni (BTI Tns5B1-4) High Five™ cell lines. G-X Li1,2, Y Hashimoto3, RR Granados1. 1Laiyang Agric. Univ., Laiyang, Shandong, China; 2Center for Biosystems Res., UMBI, College Park, MD, USA; 3Boise Thompson Inst. at Cornell Univ., Ithaca, NY, USA.

V-13 The effect of baculovirus infection on the translational machinery of lepidopteran host cells. MM van Oers1, M Doitssou1, AAM Thomas3, VM Lak1. 1Lab. of Virology, Wageningen Univ., the Netherlands; 3Dept. of Developm. Biology, Utrecht Univ., the Netherlands.

V-14 Reflex bleeding, a transmission mechanism induced by baculovirus infection in the butterfly Heliconius himea (Nymphalidae: Heliconiinae). MM Hay-Roe1. 1AM Shapiro, JJ Becnel2, DG Boucaut1. 2Dept. of Entomology and Nematology, Univ. of Florida, Gainesville, FL, USA; 3Center for Medical, Agricultural and Veterinary Entomology, USDA, ARS, Gainesville, FL, USA.

V-15 Purification and characterization of two viral particles from diseased postlarvae of Macrobachium Rosenbergii. Z Shi1, D Qian1, J-R Bonami1. 1Key Laboratory of Molecular Virology, Joint Laboratory of Invert. Virology, Wuhan Inst. of Virol., Chinese Acad. of Sci., Wuhan, China; 2Zhejiang Inst. of Freshwater Fisheries, Huzhou, China; 3UMR, DRIM, CNRS/IFREMER/UM2, Montpellier, France.


V-17 A novel envelope protein which is involved in white spot syndrome virus infection. Y Xie, R Huang, J Zhang. Z Shi. Key Lab. of Molec. Virology, Joint Lab. of Invertebr. Virology, Wuhan Institute of Virology, Chinese Acad. of Sciences, Wuhan, China.

V-18 Absence of PIF blocks baculovirus ODVs infection after the binding step. I Kikhno1, S Gutierrez2, M Ravallec1, O Simon1,2, P Caballero1, M Lopez-Ferber1. 1Lab. of Pathol. Comparée, INRA/CNRS/Univ. de Montpellier II. St Christol-les-Ales, France; 2Lab. of Entomol. Agricola y Patol. de Insectos, Depto. de Producción Agraria, Univ. Pública de Navarra, Pamplona, Spain.

V-19 Invasion process of Culex nigripalpus nucleopolyhedrovirus (CuniNPV) in midguts of larval mosquitoes. JJ Becnel1, OP Perera, A Shapiro, S White. Center for Medical, Agric. and Veterinary Entomol., US Dept. of Agric., Agric. Res. Service, Gainesville, Florida, USA.

V-20 The epithelial cell surface along the midgut of susceptible and resistant larvae of Anticarsia gemmatalis (Lepidoptera: Noctuidae) to its nucleopolyhedrovirus. SM Levy1, ÁF Falleiros1, F Moscardi1, EA Gregório1. 1Centre of Microscopy Eletrônica, IBB, UNESP, Botucatu-SP, Brazil; 2Centro de Ciências Biológicas, UEL, Londrina-PR, Brazil; 3Centro Nacional de Pesquisa da Soja, Embrapa, Londrina-PR, Brasil.

V-21 Is the Nucleopolyhedrovirus of Anticarsia gemmatalis (AgMNPy) ineffective to infect AgMNPy resistant host
BACTERIA

B-1 Endospore degradation in an asporogenic, crystalliferous mutant of Bacillus thuringiensis. P Sierra-Martinez1, JE Ibarra1, M de la Torre1, G Olmedo1,2. 1Dep. de Bacteriologia y Bioingen., Centro de Investigación y de Estudios Avanzados del IPN, Mexico, D.F.; 2Dep. de Bacteriología y Bioquímica, and 2Dep. de Ingeniería Genética, Centro de Investigación y de Estudios Avanzados del IPN, Irapuato, Gto., México.

B-2 Destruction of bacterial spores by non-contact ultrasound. K Hoover1, N Ostiguy1, M Hardway1,2. 1Dept. of Entomology, Penn State Univ., University Park, PA, USA; 2Ultrasound Laboratories, Inc., Boalsburg, PA, USA.

B-3 Laboratory and field experiments for control of Helioverpa armigera based on bitoxibacillus formulation containing Bt b–exotoxin Bt. EN Abdullah, Samarkand State Univ., Samarkand, Uzbekistan.

B-4 The research and development of BT subsp. colmeri strain 15A3 in Tianjin of China, G Ren, Y Chen, J Wang, J Cai, C Liu, B Guan. Dept. of Microbiology, College of Life Science, Nankai Univ., Tianjin, China.

B-5 Environmental distribution, frequency and diversity of Bacillus thuringiensis isolates from Spain and Latin America, CS Hernandez1, A Boets2, J Van Rie3, J Ferré2,1. 1Dep. de Genética, Univ. de Valencia, Burjassot, Spain; 2Bayer CropScience N.V., Ghent, Belgium.

B-6 Diversity of Bacillus thuringiensis strains with insecticidal activity against lepidopteran and dipteran insects. MC Escobar, G Armengol, S Orduz. Unidad de Biotecnolog, y Control Biológico, Corporación para Investigaciones Biológicas, Medellin, Colombia.


B-8 Mosquito larvicidy and synergism in transgenic Anabaena expressing four genes from B. thuringiensis subsp. israelensis, V Khasdan1,2, E Ben-Dov3,4, R Manashro3,4, S Bousis1,2, A Zaritsky3,4. 1Dep. of Life Sciences, and 2Microagial Biotechn.. Lab, Ben-Gurion Univ. of the Negev, Be’er-Sheva, Israel; 3Bio San Ltd., Ariel, Israel.

B-9 Toxicity against larvae of Aedes aegypti and synergism with Cry toxins by 3 different Cry proteins from B. thuringiensis. M Itako, R Manashro, E Ben-Dov, N Baranes, V Khasdan, A Zaritsky. Dep. of Life Sci., Ben-Gurion Univ. of the Negev, Be’er-Sheva, Israel.

B-10 Identification of two isoforms of aminopeptidase N in Aedes aegypti larval midgut. K Postanukit1, C Angsuthanumombat, S Panyim. Institute of Molecular Biol. and Genetics, Mahidol Univ., Salaya Campus, Nakhon Pathom, Thailand.

B-11 Comparative studies of Bacillus thuringiensis var. israelensis growth and spore production in different concentrations of alternative medium. S Ernandes1, K Yamaoka1, A Oshiro1, M Umsza Guez1, VL Del Bianchi1, J Van Rie2,1, D Oliveira Moraes3, 1Dep. Food Engineering and Technology, UNESP, São José do Rio Preto, Brazil; 2Univ. of Guarulhos, Guarulhos, Brazil.

B-12 Effects of Bt-transgenic potato on Podisopsis koehleri, a natural enemy of Phthorimaea operculella. J Caycho1, V Caldeyro2, A Lagounou2. 1International Potato Center (CIP) Entomology Laboratories, Lima, Peru; 2The World Bank, Environmentally and Socially Sustainable Development, Washington DC, USA.

B-13 Suitability of genetically modified Bacillus thuringiensis WG-801 for safety release on cotton fields. Z. Shu1,2, L Li1,2, M Sun1,2, Z. Yu3,4. 1Key Lab. of Agric. Microbiol., Ministry of Educ.; 2Nat. Engineer. Res. Center of Microb. Pesticides, Huazhong Agric. Univ., Wuhan, PR China.

B-14 Identification of the aminopeptidase N carbohydrate binding determinant for Bacillus thuringiensis Cry1Ac toxin. T Reyes-Izquierdo1,2, G Alvarez-Manilla1,3, M Pierce1,2, M Adang1,2. 1Centro de Investigacion en Alimentacion y Desarrollo, A.C., Hermosillo, Sonora, Mexico; 2Entomology and 3Biochemistry & Molecular Biology, Univ. of Georgia, Athens, GA, USA.

B-15 Role of HevCaLP knockout in alteration of Cry1A toxin binding in Bt-resistant Heliothis virescens strains.

B-16

Mapping the receptor binding sites on Bacillus thuringiensis Cry1Aa toxin using blocking molecules. S Atsumi, E Mizuno, M Iizuka, Y Inoue, R Sato. Grad. School of Bio-Applications & Systems Engineering, Tokyo Univ. of Agric. and Technol., Koganei, Tokyo, Japan.

B-17


B-18

Proline substitution in H4 affects helical hairpin-flexibility and membrane perturbation of the Bacillus thuringiensis Cry4B toxin. P Ounjai, G Katzmeierme, S Panyim, C Angsuthanasombat. Lab. of Molecular Biophysics, Inst. of Molecular Biology and Genetics, Mahidol Univ., Salaya Campus, Thailand.

B-19

Characterization of the cloned Cry4B domain III fragment. P Chayaratatanasin, C Pothiranata, G Katzmeierme, S Panyim, D Gerber, Y Shai, C Angsuthanasombat. 1Laboratory of Mol. Biophysics, Inst. of Molecular Biol. and Genetics, Mahidol Univ., Salaya Campus, Nakornpathom, Thailand; 2Dept. of Biological Chemistry, Weizmann Inst. of Science, Rehovot, Israel.

B-20

Bacterial male-killers: inherited symbionts with a cutthroat strategy. MEN Majerus, HE Roy. 1Dept. of Genetics, Univ. of Cambridge, Cambridge, UK; 2Dept. of Life Sciences, Anglia Polytechnic Univ., Cambridge, UK.

B-21

Maximizing the use of mass spectrometry data generated from proteomic analyses of insects with relatively few sequenced proteins. RJ McNall, MJ Adang. 1Biochemistry & Molecular Biology and "Entomology, Univ. of Georgia, Athens, GA, USA.

B-22

Analysis of midgut brush border proteins in Bt susceptible and resistant Plutella xylostella larvae using differential two-dimensional electrophoresis. RJ McNall, MJ Adang. 1Biochemistry & Molec. Biology and "Entomology, Univ. of Georgia, Athens, GA, USA.

B-23

Interaction of Bacillus thuringiensis toxins with Helicoverpa armigera midgut. A Estela, J Ferré, B Escribè. Dept. of Genetics, Faculty of Biology, Univ. of Valencia, Burjassot, Valencia, Spain.

B-24

Identification of the western spruce budworm midgut receptor for Bacillus thuringiensis insecticidal Cry toxins. AP Valaitis, USDA Forest Service, Northeastern Res. Sta., Durham, NH, USA.

B-25

Wolbachia in sucking lice. G Kyei-Poku, DD Colwell, P Coglin, KD Float. Lethbridge Research Centre, Agriculture and Agri-Food Canada Lethbridge, Alberta, Canada.

B-26

Molecular evidence and phylogenetic relationships of Wolbachia infection in wasps parasitic on pest flies affecting livestock. G Kyei-Poku, K Floate, B Benkel, MS Geiit. Lethbridge Res. Centre, Agric. and Agri-Food Canada, Lethbridge, Alberta, Canada.

B-27

Adenyl cyclase and protein kinase A affected the hemocytes-mediated responses of Malacosoma disstria to Xenorhabdus nematophila and Bacillus thuringiensis. V Galli, CL Brooks, GB Dunphy. Dept. of Natural Resource Sciences, McGill Univ., Montreal, Quebec, Canada.

B-28


B-29

Endoparasitic nematodes as targets of nematicidal Bt crystal proteins in transgenic plants. X Li, S Parsa, RV Aroian. Sect. of Cell Develop. Biology, Univ. of California, San Diego, CA, USA.

B-30

Bacterial male-killers: inherited symbionts with a cutthroat strategy. MEN Majerus, HE Roy. 1Dept. of Genetics, Univ. of Cambridge, Cambridge, UK; 2Dept. of Life Sciences, Anglia Polytechnic Univ., Cambridge, UK.
9:20 Managing an unwanted visitor at Acadia National Park. E Groden, F. Drummond, S. Yan. Dept. of Biological Sciences, Univ. of Maine, Orono, Maine, USA.


9:45 Expression and purification of an active superoxide dismutase from Amsacta moorei entomopoxvirus (AmEPV). MN Becker1, A Bawden1, D Aramburo2, W Greenleaf1, R Moyer1. Depts. of Molecular Genetics and Microbiology, and Pharmacology, Univ. of Florida, Gainesville, FL, USA.

8:45 Use of dsRNA to generate transgenic silkworms resistant to BmNPV. R Sober1, T Matsuyama1, K Kojima1, T Kanda1, T Tamura2, K Sahara2, S Asano1, H Bando1. 1Div. of Mol. Genetics and Microbiol., Univ. of Florida College of Medicine, Gainesville, FL, USA; 2Dept. of Entomol., Connectiicut Agric. Extpt. Station, New Haven, Connecticut, USA.

8:30 The ADP-ribosylating mosquitocidal toxin (MTX) from Bacillus sphaericus SS1-1. J Schimirer, J Caprusca, K Aktories. Dept. of Experimental and Clinical Pharmacology and Toxicology, Univ. of Freiburg, Freiburg, Germany.

9:00 Membrane permeabilizing activity of the 70 kDa moiety of the Mtx toxin from Bacillus sphaericus. J-L Schwartz1,2, A Maria Gariria Rivera1, L Potvin3, C Berry3 G Menestrina4. 1Biotechnology Research Institute, National Research Council, Montreal, Que., Canada; 2GÉPROM and Biocontrol Network, Univ. de Montréal, Que., Canada; 3Cardiff School of Biosciences, Cardiff Univ., Cardiff, UK; 4CNR-ITC, Centro di Fisica degli Stati Aggregati, I-38050 Povo, Italy.

9:30 Comparative analysis of baculovirus envelope fusion protein F and a cellular F homolog of D. melanogaster. O. Lung1, G.W. Blissard2. Boyce Thompson Inst. at Cornell Univ., Ithaca NY, USA.

10:00–10:30 BREAK Green Mt. atrium

12:30–2:00 LUNCH Adirondack Ballroom

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

3:15 Modeling Nosema disease in honey bee colonies. O. Lung1, G.W. Blissard2. 1Dept. of Entomol. & Biodiversity, Univ. of Adelaide, Australia; 2Boyce Thompson Inst., Ithaca, NY, USA.

3:40 Panel discussion.
Symposium (Div. of Bacteria) Wednesday, 2:00-3:00. Green Mt.–C

Mode of action of bacterial toxins (Part 2)

Organizers/Moderators: David Ellar, Alejandra Bravo.
Moderator: Alejandra Bravo.


2:30 Toxins from Xenorhabdus species. A. Morgan.

Symposium (Div. of Bacteria) Wednesday, 3:00-4:00. Green Mt.–C

Mode of action of three-domain Cry toxin family (Part 1)

Organizers: David Ellar, Alejandra Bravo.
Moderator: A. Bravo.

3:00 Mapping Binding Epitopes on Cry Proteins. M.A.F. Abdullah1, A. White1, R.J. McNall1, M.J. Adang2, D.H. Dean1. 1Dept. of Biochem., The Ohio State Univ., Columbus, OH, USA; 2Dept. of Entomol., Univ. of Georgia, Athens, GA, USA.

3:30 Receptors and rafts in Cry toxin action. M. Zhuang1,2, R. Xie1,2, I. Gomez1, M. Soberón1, A. Bravo1, L.S. Ross1, S.S. Gill1. 1Graduate Program in Envir. Toxicology, 2Dept. of Cell Biology and Neuroscience, Univ. of California, Riverside, CA, USA; 3Instituto de Biotecnología, Depto. de Microbiología, Univ. Nacional Autónoma de México, Cuernavaca, Morelos, México.

Contributed Papers Wednesday, 2:00-4:00. Lake Champlain

VIRUSES – 5

Moderator: Ping Wang.


2:15 Molecular mechanism for HaNPV transporting to the host nucleus. S. Lu, Y. Qi, G. Ge. College of Life Sci., Wuhan Univ., Wuhan, Hubei, 430072, P.R.China.


3:00 Providence virus: a new tetravirus with an unusual arrangement of its non-structural genes. E.M. Pringle, K.N. Johnson, L.A. Ball. Univ. of Alabama at Birmingham, Dept. of Microbiology, Birmingham, AL, USA.


Symposium (Div. of Viruses) Wednesday, 4:30-6:30. Green Mt.–A

Baculovirus genomics

Organizers/Moderators: James Maruniak, David Theilman.


4:30 Interaction of Cry1A toxin with BtR1 and its role in a pre-pore formation. I. Gómez, C. Rausell, C. Muñoz-Garay, A. Bravo, M. Soberón. Instituto de Biotecnología UNAM, Cuernavaca, Morelos, México.


5:18 Complete genome comparison of two baculoviruses that are highly pathogenic for the cabbage looper; Trichoplusia ni single nucleopolyhedrovirus (Group II NPV) and Autographa californica nucleopolyhedrovirus (Group I NPV).

5:42 Analysis of molecular adaptation of nucleopolyhedrovirus genes. R.L. Harrison, B.C. Bonning. Dept. of Entomol. and Interdepartmental Program in Genetics, Iowa State Univ., Ames, Iowa USA.


6:06 Analysis of molecular adaptation of nucleopolyhedrovirus genes. R.L. Harrison, B.C. Bonning. Dept. of Entomol. and Interdepartmental Program in Genetics, Iowa State Univ., Ames, Iowa USA.

5:18 Analysis of molecular adaptation of nucleopolyhedrovirus genes. R.L. Harrison, B.C. Bonning. Dept. of Entomol. and Interdepartmental Program in Genetics, Iowa State Univ., Ames, Iowa USA.


6:06 Complete genome comparison of two baculoviruses that are highly pathogenic for the cabbage looper; Trichoplusia ni single nucleopolyhedrovirus (Group II NPV) and Autographa californica nucleopolyhedrovirus (Group I NPV).
Contributed Papers Wednesday, 4:30-6:30. Lake Champlain

**FUNGI – 3**

**Moderator: Melanie Filotas.**

- **4:30**  

- **4:45**  
  Molecular mechanisms of adaptive radiation in *Metarhizium anisopliae*. R. St. Leger, G. Hu. Dept. of Entomology, Univ. of Maryland, College Park, MD, USA.

- **5:00**  
  A multigene phylogeny of *Beauveria*: new insights into species diversity, biogeography, host affiliation and life history. S.A. Rehner. USDA-ARS, Insect Biocontrol Lab., Beltsville, Maryland 20705, USA.

- **5:15**  
  Phylogenetic and population genetic approaches to the analysis of cryptic speciation in the *Beauveria bassiana* s.str. complex. S.A. Rehner. USDA-ARS, Insect Biocontrol Lab., Beltsville, Maryland 20705, USA.

- **5:30**  
  Risk assessment of using mycoinsecticides: Prevalence of a commercial *Beauveria bassiana* strain and its impact on conspecific indigenous populations. L.A. Castrillo¹, E. Groden¹, S.L. Annis¹, J.D. Vandenberg. Dept. of Entomol., Cornell Univ., Ithaca, NY, USA; ²Dept. of Biol. Sciences, Univ. of Maine, Orono, ME, USA; ³USDA-ARS, US Plant, Soil & Nutrition Lab., Ithaca, NY, USA.

- **5:45**  
  Evaluation of entomopathogenic fungi for microbial control of the greenhouse pests *Myzus persicae* and *Aphis gossypii*. M Filotas¹, S Wraight², J Sanderson². ¹Dept. of Entomology, Cornell Univ., Ithaca, NY, USA; ²USDA Agriculture Research Service, US Plant, Soil, & Nutrition Lab., Ithaca, NY, USA.

- **6:00**  
  The effects of drying on germination and activity of *Metarhizium anisopliae* var. *acridum* conidiospores. B.P. Magalhães¹,², D.G. Boucias¹. ¹Entomol. & Nematol. Dept., Univ. of Florida, Gainesville, Florida, USA; ²Permanent address: Embrapa Recursos Genéticos e Biotecnologia, Brasília, DF, Brazil.

- **6:15**  
  Potential use of *Paecilomyces fumosoroseus* for control of the Formosan subterranean termite, *Coptotermes formosanus* Shiraki. M.S. Wright¹, M.A. Jackson¹, W.J. Connick¹. ¹USDA, Agric. Research Service, Southern Regional Research Center, New Orleans, LA, USA; ²USDA, Agric. Research Service, National Center for Agric. Utilization Research, Peoria, IL, USA.

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**7:30p–9:00m**  
**BANQUET**  
Adirondack Ballroom

**AWARDS CEREMONY**

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WE HOPE TO SEE YOU IN 2004 IN HELSINKI!
IMPORTANT NOTES:
These abstracts should not be considered to be publications and should not be cited in print without the author’s permission.

STU indicates papers being judged for graduate student presentation awards
The animal intestine as the organ for ingestion and digestion of food has often been a target of food borne pathogens. To avoid being digested, successful pathogens have evolved many strategies including escaping from the intestine as soon as possible. The mosquito intestine, like that of other insects, is composed of a monolayer of epithelial cells held together by a grid of muscle bundles and layers of extracellular matrix proteins. Therefore, it acts as a physical and biological barrier to all ingested pathogens. Malaria parasites undergo developmental changes in the posterior midgut of the mosquito intestine and differentiate into a slender, motile ookinetes. Studies with the avian parasite *Plasmodium berghei* invasion of *Anopheles stephensi* midgut, *P. gallinaceum* ookinetes preferentially invade a particular cell-type with distinct histological and ultrastructural features in *Ae. Aegypti* midgut. These cells also express a higher level of vesicular ATPase. The preferential invasion by the avian parasite to a particular cell type is corroborated by the multiple invasion of a midgut cell by more than one ookinete. The correlation between the distribution of the V-ATPase positive cell type to the posterior midgut and the distribution of the oocysts on midgut surface also supports this notion. Unlike what was reported for *P. berghei* invaded midgut cells in *An. stephensi*, nitric oxide synthase does not appear to be induced in *P. gallinaceum* invaded *Ae. aegypti* midgut cells. A robust blood meal induced nitrosylation of tyrosine residues, unrelated to parasite invasion, is observed in some *Ae. aegypti* midgut cells about 18 hours after a blood feeding which begins to subside around 30 hours after the feeding. To examine the role of the midgut invasion in determining the susceptibility to the parasite, *P. gallinaceum* infected blood was fed to a refractory strain of *Anopheles gambiae* and the rate of ookinete invasion was found to be similar to that in susceptible *Ae. aegypti* midgut. However, most invaded parasite appeared to be destroyed in the midgut cell cytoplasm of the refractory *An. gambiae*, suggesting that major attrition of *P. gallinaceum* ookinete in this refractory mosquito occurs after invasion. We will discuss a model for ookinete invasion of mosquito midgut epithelium and examine how this may help understanding interactions of human malaria parasite with vector midgut before its development as an oocyst and infectious sporozoites.

**Arbovirus-vector interactions in midguts of the mosquito, *Aedes aegypti*"
In order to generate fatal systemic infections, occlusion-derived virions (ODV) of AcMNPV, the type species of the Baculoviridae, must first generate productive, primary infections within midgut cells of their lepidopteran hosts. The midgut is also the host’s first line of defense and poses a formidable barrier to infection. In addition to having harsh digestive juices within their gut lumen, the larvae of many host species are able to recognize and slough ODV-infected midgut cells. Moreover, in most hosts, all ODV-infected midgut cells are lost during molting when a new and larger midgut tissue is differentiated for the subsequent instar. Sloughing responses are variable, both within and among species, and they are the principal reasons why hosts exhibit developmental resistance. AcMNPV has a remarkably broad host range, and many of its hosts (e.g., Trichoplusia ni, Heliothis virescens, Spodoptera exigua, and Spodoptera frugiperda) have little, if any, systemic defense once BV has entered the hemocoel. Not surprisingly, over evolutionary time, selection has favored an AcMNPV infection strategy that counters the midgut cell sloughing response of its hosts. This strategy involves two traits, packaging of multiple nucleocapsids within ODV and early expression of the essential BV structural protein GP64. Together, these traits enable ODV nucleocapsids to bud from infected midgut cells, essentially as BV, and establish secondary infections within tracheal epithelial cells prior to completion of viral replication within the midgut. The effects of early GP64 synthesis on AcMNPV oral virulence in developmental cohorts of H. virescens, S. exigua and T. ni are variable and modulated by at least three factors: 1) host sloughing, 2) the temporal onset of primary infections, and 3) the number of midgut foci generated per occlusion. By contrast, in S. frugiperda larvae, the principal midgut barrier to infection is the inability of AcMNPV ODV to infect midgut epithelial cells, possible due to the lack of binding to an appropriate receptor. In all hosts studied to date, AcMNPV infects the midgut only transiently. This allows the tissue to continue functioning during the protracted time required for BV to spread throughout the host. As a result, the host continues to feed and grow, accumulating biomass that ultimately can be utilized to enhance the production of viral progeny.

**POST-PLENARY SYMPOSIUM. Sunday, 2:00-4:00.**

**New approaches for studying toxicity, infection and pathogenesis**

_Symposium. Sunday, 2:00_

_Carol D. Blair, Emily E. Travanty, Irma Sanchez-Vargas, Kimberly M. Keene, Ken E. Olson, and Barry J. Beatty_

**Arthropod-borne and Infectious Diseases Laboratory, Dept. of Microbiology, Immunology, and Pathology, Colorado State Univ., Fort Collins, Colorado 80523, USA**

Arthropod-borne virus diseases are increasingly significant, global human and animal health problems, and novel methods are needed to control their transmission. Post-transcriptional gene silencing triggered by double-stranded (ds) RNA has been described in plants and a number of invertebrates, and, at least in plants, is thought to serve as an anti-viral defense mechanism. We have exploited a gene silencing phenomenon called RNA interference (RNAi) to render mosquitoes resistant to infection with and incapable of transmission of dengue virus type-2 (DV2; Flaviviridae). We have shown that expression of a portion of the DV2 RNA genome in cells of mosquito midguts and salivary glands triggers resistance to subsequent infection by a homologous virus. Expression of either positive or negative sense RNA can elicit RNAi, although expressed dsRNA is the most effective trigger, and RNAi is sequence specific. The hallmark of RNAi, small interfering (si) RNA 20-23 nucleotides in length with sequence homology to the dsRNA trigger, can be demonstrated in invertebrate cells. We have now shown that arboviruses from at least 3 virus families induce the production of siRNA upon infection of mosquito cells, and have identified genes putatively required for RNAi in both Anopheles gambiae and Aedes aegypti. Both quantitative and temporal expression of siRNA and the apparent effect on the replicative capacity of arboviruses vary in different virus-host systems, and thus we hypothesize that the balance between the host’s ability to mount an RNAi defense and the potential for viruses specifically to suppress interference are determinants of viral persistent infections in arthropod vectors.

**Function of the peritrophic membrane in viral pathogenesis**

_Ping Wang_

_Dept. of Entomology, New York State Agricultural Experiment Station, Cornell Univ., Geneva, NY 14456, USA_

Insect midgut is the primary site interfacing with various challenging environmental factors and is the major portal of entry for microbial pathogens. The midgut is commonly lined by an invertebrate unique chitin-protein structure, the peritrophic membrane (PM). The PM plays multiple functions, including compartmentalizing the midgut lumen, assisting digestion and protecting the midgut epithelium from physical and chemical damages and biological infections. To understand the structure and formation of the PM and its protective function in viral pathogenesis in insects, we studied the PM proteins in a lepidopterous species, Trichoplusia ni. In T. ni larvae, the PM lines the midgut epithelium for the entire active feeding stage. The protein composition of the PM is complex and the majority of the proteins are chitin binding proteins. The high affinity binding of the PM proteins to PM chitin fibrils appears to be an important mechanism for the PM structural formation. Both mucin and non-mucin PM proteins were identified and their biochemical and molecular characteristics were studied. Our studies suggested a molecular model indicating the unique structural features of PM proteins for the PM formation and function. Functional analysis of the PM clearly indicates that the PM plays an important role in protecting the midgut epithelium from viral infection.

**Beet armyworm midgut gene expression correlated with sensitivity or resistance to Bacillus thuringiensis delta-endotoxin Cry1Ca**

_Ruud A. de Maagd1, Petra L. Bakker1, Tsanko Gechev2, Tjie-Yien Man3, Salvador Herrera3, and William J. Moar2_

1Plant Research International, Wageningen, The Netherlands; 2Dept. of Entomology, Auburn Univ., Auburn, AL 36849, USA_

We are studying molecular processes in the midgut of beet armyworm (Spodoptera exigua) larvae that may be involved in overcoming damage following intoxication by a Bt toxin, or in resistance to such a toxin. For this purpose we have made libraries of cloned cDNA fragments representing genes that are differentially expressed in larvae grown on a sublethal dose of Cry1Ca. Libraries were made using Suppression Subtractive Hybridization (SSH) to enrich for genes that are either lower or higher expressed in toxin-exposed versus non-exposed larvae. These cDNAs were used to produce cDNA microarrays on glass slides for gene expression studies. We followed the expression of these genes under different conditions of toxin exposure (variations in dose, time of exposure, non-active toxin mutants) in Cry1Ca-sensitive larvae as well as in larvae of a laboratory-selected Cry1Ca-resistant colony. Most of the cDNAs representing up-regulated genes have no significant homology with other proteins in public databases. Most prominent is a set of 4 fast, strongly-upregulated genes encoding homologous proteins (40-80% amino acid identity) of 136 amino acids including a putative signal sequence. Down-regulated in response to toxin exposure are mostly genes with homology to lipid hydrolases (lipases, triacylglycerol-hydrolases). The vast majority of gene expression differences were clearly...
correlated with toxic action of Cry1Ca, as they did not occur with inactive other toxins or Cry1Ca-mutants, and occurred in resistant larvae only at much higher doses. Selected gene expression differences were confirmed using RT-PCR.

SSH proved very successful at isolating cDNAs for genes that are differentially expressed from two pools of mRNA. For this reason we applied the same technique to pools of midgut mRNA from Cry1Ca-sensitive versus resistant larvae, in order to detect gene expression differences that may be involved in the mechanism of resistance to Cry1Ca. This has lead to the identification of a putative Cry1Ca-receptor that is not expressed in larvae of the resistant colony.

Symposium. Sunday, 3:12

Proteomic analyses of Bacillus thuringiensis toxin – insect midgut interactions

Michael J. Adang1,2 and Rebecca J. McNall1

1Biochemistry & Molecular Biology and 2Entomology, Univ. of Georgia, Athens, GA 30602

We will discuss proteomic approaches to investigating the complex mechanism of Bacillus thuringiensis (Bt) Cry toxin action. Proteomic analyses allow the large-scale examination of proteins bypassing the more traditional need to purify individual proteins. Bt Cry proteins disrupt the midgut epithelium of susceptible larvae. A simplistic summary of this process follows. Toxin binds receptor proteins, undergoes a conformational rearrangement and inserts into brush border membrane forming pores resulting in cellular death. Known receptor proteins include aminopeptidase N (APN), cadherin-like proteins and glycoconjugates. Insects can become resistant to Bt toxins, and the most common mechanism of resistance is loss of binding to the midgut brush border membrane.

In the first study, we validated a proteomic approach using Manduca sexta BBMV and Cry1Ac toxin. A subset of GPI-anchored proteins was also examined. BBMV proteins were separated by two-dimensional gel electrophoresis (2DE) followed by staining or blotting to membrane filters. Blots were probed with Cry1Ac or antibody against GPI-anchored proteins. Peptide mass fingerprints (PMF) were generated for selected spots from 2DE gels. Using web-based bioinformatics programs, fingerprints were compared to in silico-digested peptides in databases yielding potential matches. To confirm search results western blotting was performed. Actin, APN, and membrane alkaline phosphatase were confirmed as accurate protein identifications for Cry1Ac-binding proteins.

In the second study, we compared proteins from Bt-susceptible Plutella xylostella with proteins from resistant larvae using fluorescence 2DE. Resistant larvae tolerate high doses of Cry1A and Cry1Fa toxins and are characterized by a Type 1 resistance mechanism. BBMV from resistant larvae have greatly reduced binding of Cry1a and Cry1Fa toxins. Brush border proteins of susceptible and resistant larvae were labeled with different fluorescent dyes then separated by 2DE. Protein spots altered in resistant larvae were subjected to PMF and matched to proteins in databases as described above. We will also discuss current limitations and speculate on additional applications of proteomic technologies to examining pathogen–insect interactions.

Symposium. Sunday, 3:36

Tracking the infection process of Bacillus thuringiensis in the insect

Christina Nielsen-LeRoux1,2, Christophe Buisson1, Patricia Nel1, Myriam Hajaij1, Sinda Fedhila1, Elisabeth Guillemet1, Laurence Fiette1, and Didier Lereclus1,2

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The main insecticidal activity of Bacillus thuringiensis (Bt) is due to the larval ingestion of the insect specific Cry toxins. However, both Bt crystal mutant and B. cereus strains are known to produce other factors contributing to the overall virulence of these bacteria toward insect, mice and in some cases to man. For both species, injection of spores or vegetative cell into the insect hemolymph is highly pathogenic. Moreover, synergy between spores and sublethal doses of Cry toxin is observed when insects, weakly susceptible to Cry toxins, are infected orally. The importance of the Bt pleiotropic PlcR regulator was demonstrated by reduced mortality in Galleria mellonella from spores of a Bt 407 [Cry] plcR mutant [1] PlcR controls the expression of many putative virulence factors (phospholipases, enterotoxins, hemolysins, proteases etc.), and recently the putative zinc protease InhA2 was shown to be important for pathogenicity via the oral route [2,3]. InhA2 may interfere with intestinal barriers (peritrophic membrane and/or intestinal midgut cells). In order to improve the understanding of bacteria–host cell interactions, particularly in the digestive tract, we investigated the localization of Bt cells during the infection process conducting to septicaemia. Eventual indications for “blocking levels” of attenuated mutants and identification of target tissues for virulence factors, are also anticipated. In vivo visualization of the oral infection process is made possible by the use of plasmids carrying transcriptional fusions between the gfp gene (green fluorescence protein) and the promoters of Bt (PlcA, Apha3, Cry 1) which are known to be activated at different bacterial growth stages. Spores from Bt 407 [Cry] wild type, PlcR and inhA2 mutants, carrying these plasmids, were used in oral infection alone or with a sublethal dose of Cry1C. Infection kinetics were recorded by fluorescence microscopy and by histopathological observations, directly or on fixed forced fed 5th instar Galleria mellonella. [1]Salamyto S. et al. 2000 The regulator PlcR is involved in the opportunistic properties of Bacillus thuringiensis and Bacillus cereus in mice and insects. Microbiology 146:2825-2832. [2]Fedhila, S., Nel, P., Lereclus, D., 2002. The InhA2 metalloprotease of Bacillus thuringiensis strain 407 is required for pathogenicity in insects via the oral route. J. Bacteriol. 184:3266-3274. [3]Fediha, S., Gohar, M., Slami, L., Nel, P., Lereclus, D., 2003. The Bacillus thuringiensis PlcR-regulated gene inhA2 is necessary, but not sufficient, for virulence. J. Bacteriol. In press.

SYMMPOSIUM (Division of Fungi). Sunday, 2:00-4:00.

Conservation microbial biocontrol

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For the past 14 years the cotton aphid fungus, Neozygites fresenii, has been the most important natural enemy of the cotton aphid, Aphis gossypii, across the cotton growing regions of the Midsouth and Southeast United States. Each year this fungus terminates aphid populations by causing epizootics that occur over a 4 week period in June and July over millions of hectares of cotton. The importance of conservation and utilization of this pathogen in cotton production is well recognized by growers, consultants, scientists and extension agents. To utilize and conserve this pathogen a service was established, now in its 11th year, to sample and diagnose cotton aphids across the southern United States. Aphid samples are collected from cotton fields throughout the season. The prevalence of N. fresenii in the aphid population is determined by diagnosis of the aphid samples. When diagnoses indicate that the fungus is active in a field or area, chemical treatments are not made for the aphids, conserving the fungus and allowing it to spread through and reduce the aphid population. The value each year of control provided by N. fresenii to cotton growers is estimated at $30 million. Naturally-occurring entomopathogens are major factors in reducing or controlling many insect populations. Some examples of pathogens that cause annual epizootics in pests of major crops are: Neozygites floridana in spider mites, Nomuraea rileyi in velvetbean caterpillars, and Zoophthora phytonomi in alfalfa weevil. Kish and Allen (1978) proposed a program for predicting incidence of N. rileyi on velvetbean caterpillars on soybean. To our knowledge, the cotton aphid fungus sampling program remains one of the more successful efforts to utilize and conserve a natural entomopathogen in a major crop.
Managed field margins as refugia for *Pandora neoaphidis*

P.A. Shah and J.K. Pell

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Conservation microbial control is defined as the enhancement of pathogen impact without the release of entomopathogens into an environment. Fungi belonging to the order Entomophthorales are well suited to exploitation in this strategy against pest arthropods. In the UK, sowing of non-crop plants in strips adjacent to crops is being encouraged under agri-environment schemes. At Rothamsted we are evaluating the potential for these margins to be managed as refugia for *Pandora neoaphidis*, and other aphidophagous Entomophthorales, which would act as a source of infection to disperse into pest aphid populations in adjoining crops.

In laboratory bioassays, the pea aphid, *Acyrthosiphon pisum*, was highly susceptible to *P. neoaphidis*, while the cereal aphid, *Rhopalosiphum padi*, was least susceptible. However, most isolates -including those from non-pest aphids (potential margin species) - were able to infect pest aphids. Molecular analyses of *P. neoaphidis* isolates obtained from UK and elsewhere revealed three clusters which could not be related to geographical origin or aphid host, and suggests mobility of isolates between hosts.

Sampling to determine *P. neoaphidis* spatio-temporal dynamics has been carried in an experimental field margin and adjacent wheat crop. Although aphid populations have been low, the ratio of living to infected aphids has remained high. A further experiment is being carried out in a margin planted with non-crop plants in a field margin in an agri-environment scheme. In this study, *P. neoaphidis* isolates obtained from field collected aphids will be evaluated. The project is being further extended, particularly in South Africa, where *P. neoaphidis* is also being evaluated for potential control of invasive *Parthenium hysterophorus*, as well as other invasive species such as *Eupatorium*.

Overall, these studies demonstrate that *P. neoaphidis* could be exploited for conservation microbial control of aphids using field margins, but further research is needed to develop the strategy and implement it at a farm scale.

Symposium, Sunday, 2:30

Hedgerows, flies, aphids and winter survival of Entomophthorales

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The initiation of infection in insect populations after the winter is among the main events to determine if fungi from the Entomophthorales will develop epizootics in insect populations and by that contribute to conservation biological control. We have studied two different systems of host-pathogen interactions with particular reference to the winter survival of the fungi:

1. Adult Diptera (Delia spp., *Pollenia* spp. and others) – *Entomophthora muscae, Entomophthora schizophaeae* and *Strongwellsea* spp.
2. Aphids (*Sitobion avenueae*) – *Pandora neoaphidis*

The life-cycle of host pathogen interactions of each of these will be discussed in relation to winter survival of fungal structures and the importance of landscape elements for this survival. Further, we discuss how it might be possible to operationalize the conservation biological control in these systems.

Symposium, Sunday, 3:00

Contribution of potential biocontrol agents against adventive species of *Lymantria dispar L.*


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Microsporidia pathogenic to the gypsy moth, *Lymantria dispar L.*, have been recovered from seven European countries since 1994 but do not occur in North American gypsy moth populations. The isolates represent three different genera, *Nosema*, *Vairimorpha*, and *Endoreticulatus*, and are potential candidates for introduction as classical biological control agents against *L. dispar* in the U.S. Several species were previously described but problems with early descriptions, particularly within the *Nosema* group, need to be resolved and the remaining isolates identified. The taxonomic determination and characterization of these isolates is essential if they are to be considered for importation. We evaluated the use of 2-dimensional polyacrylamide gel electrophoresis (2D-PAGE) to produce protein patterns reflecting the activity of genes between closely related species or biotypes of the microsporidia. We developed a method for solubilizing proteins from microsporidian spores that produces approximately 200 isolated protein spots on gels stained with SYPRO Ruby protein stain (Bio-Rad). We compared three microsporidian isolates for which the small subunit rDNA sequences are known, two *Nosema* isolates and one closely related *Vairimorpha* isolate that differs from the *Nosema* group by 2-3 bp. All isolates were produced and assessed under identical conditions in *L. dispar* larvae. The protein expression patterns revealed that, for each species, several proteins were found to be unique in their molecular weight, isoelectric points and intensity of staining. Spot correlation was higher between the two *Nosema* isolates than between either isolate and *Vairimorpha*. In addition, differences between the *Nosema* isolates suggest that this method can be used to evaluate relative differences between microsporidian biotypes that would facilitate studies of taxonomy, speciation and host specificity.

Symposium, Sunday, 3:30

Conservation of natural enemies of weeds and plant pathogens

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Classical biological control, or to use current jargon the “enemy release theory”, is a well-established and often highly successful practice for the management of invasive alien weeds. This approach is also being evaluated for potential control of invasive plant diseases. However, the classical tactic appears to have been under-exploited in insect pathology despite its long history of involvement in biological control. The central tenet of the theory is that the best or most efficient biocontrol agents occur in the centre of origin or diversity of the alien target pest since the natural enemies found there have coevolved with their host. This paper tests the hypothesis, based on example from plant pathology, focusing on highly invasive alien weeds and plant pathogens which, paradoxically, are rare to threatened in their native ranges and for which their conservation has been or is proving to be essential for successful implementation of classical biological control. One such example is mistflower weed (*Ageratina riparia*), a troublesome invasive species in both natural forest ecosystems and upland pastures in Australia, Hawaii, New Zealand and South Africa, which is so rare in its area of origin, in the mountainous canyons of Veracruz State, that no specimens could be located in Mexican herbaria, and the plant was unknown to local botanists. A white smut fungus found in the type locality had not successfully controlled the weed in Hawaii and South Africa, and has recently been released in New Zealand. Another on-going project has managed to trace the natural forest host of a highly damaging and increasingly invasive pathogen of the cocoa crop in Latin America, which occurs in a biodiversity “hot spot” on the western slopes of the Ecuadorian Andes. Fungal natural enemies, isolated from both naturally-diseased and healthy pods of a rare endemic *Theobroma* species, have proven to be significantly more diverse and efficient in controlling the disease in greenhouse screening experiments when compared to non-coevolved mycoparasites isolated from diseased cocoa pods in the invaded range, including strains of the same species. Such evidence adds support to the “enemy release theory” and, in addition, highlights the need to conserve these natural ecosystems which are sources of potential biocontrol agents.

Symposium, Sunday, 2:00-4:00
Is permissiveness of *Lymantria dispar* larvae to microsporidian infections determined by the host's immune response?

**Gernot Hoch**1,2, Leffen L. Sotler1 and Axel Schöpf2

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*Lymantria dispar* larvae can be infected in the laboratory with a variety of microsporidia isolated from other lepidopteran hosts. The larvae, however, are frequently semi-permissive hosts for these pathogens, while microsporidia naturally occurring in *L. dispar* cause heavy infections. We studied the extent to which the immune response of the host could determine its permissiveness for microsporidian infections. We analyzed phenoloxidase activity and the total number of hemocytes in the hemolymph of *L. dispar* larvae following infections with naturally occurring microsporidia as well as with microsporidia from other lepidopteran hosts. Most infections elicited an activation of the prophenoloxidase system. Tissue specificity and intensity of the infections, rather than host permissiveness determined the level of the activation. Heavy infections of the fat body induced the highest phenoloxidase activity while infections primarily of the silk glands elicited a lower activity. Infections of gut tissues or very light infections were not accompanied by elevated phenoloxidase activity. With the exception of one *Vairimorpha* sp. from *L. dispar*, most infections we studied led to a slight, temporary decrease in hemocyte numbers. In a second step we employed 'pseudoparasitization' by gamma-irradiated baculovirus particles to suppress the immune system in order to analyze possible alterations in the course of the various microsporidian infections. Pseudoparasitization led to slight, but often significant increases in the number of spores produced per host. This trend existed for all studied microsporidia, but the parasitoid altered host suitability in a rather subtle way; neither the course of the infections nor the tissues invaded were modified. Overall, we conclude that it is not primarily the immune response of *L. dispar* that determines its permissiveness for various microsporidia, rather, observed defense responses seem to be induced by damage of tissues due to heavy infections.

**STU** Contributed paper. Sunday, 2:30

**Factors affecting transmission of the microsporidian, Nosema fumiferanae, a natural pathogen of the spruce budworm**

**Christina Campbell**1, Sandy Smith1, and Kees van Frankenhuyzen2

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The spruce budworm (Lepidoptera: Tortricidae) is a major forest pest throughout the boreal forests of North America. During outbreaks, extensive feeding on spruce and fir foliage results in massive tree mortality and economic loss. The cyclic nature of budworm populations may be driven by natural biotic factors. A parasitic protozoan, *Nosema fumiferanae* (Microsporida: Nosematidae), is often found at high levels during a budworm outbreak and delays budworm development and reduces fecundity. This sublethal pathogen is thought to be involved in the collapse of budworm populations. The role of *Nosema* in budworm population dynamics is complicated by two means of transmission: *per os* (horizontal) and transovarial (vertical).

In the current paper, I explore the rate of horizontal transmission and *spore production* of *Nosema* in the spruce budworm by examining differing levels of infection, larval instars and temperature. To mimic infection at different budworm densities, I will rear larvae in five infection ratios (infected to uninfected individuals). These ratios will provide information on how the pathogen spreads and can reach high infection levels during budworm outbreaks. Efficiency and the rate of vertical transmission will also be determined.

My work will expand our current state of knowledge about insect-pathogen population dynamics in general, and help integrate information on disease transmission with current management plans for budworm monitoring and control. Most insect literature focuses on lethal pathogens and the involvement of sublethal pathogens in the system has largely been ignored.

**STU** Contributed paper. Sunday, 2:45

**Modelling the transmission of an insect pathogen (Microsporidia) on its host, Lymantria dispar**

**Dörte Goertz**1,2, David Onstad2, David Crowder2, Andreas Linde1

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Microsporidia not known in North American Gypsy Moth, *Lymantria dispar*, populations infect European populations of the same host and caused several times a break down of outbreak populations. Therefore different microsporidian isolates were sampled in an international cooperation during the last years. We performed several laboratory studies investigating the bionomical effects of an infection on Gypsy Moth larvae and small laboratory populations, and tested the horizontal and vertical transmission of these pathogens. Our results showed sublethal effects of the microsporidia such as prolonged development, moderate mortality, reduced fecundity and hatch of the progeny. In further experiments we focused on the possible pathways of transmission because of variable results in transmission efficiency.

To get a better understanding of our lab studies we constructed a simulation model of disease situations, using the basic ideas of ANDERSON AND MAY (1981) and modifications by DWYER ET AL. (2000). Important features of our model were the seasonal development of the Gypsy Moth (DWYER ET AL., 2000), the latency period occurring after an infection with microsporidia and two different transmission pathways. The results of our simulation experiments show the importance of the latency period and the duration of larval stage of susceptible hosts as well as the larval and pupal mortality of infected hosts. The latency periods and the lengths of larval stage, found in our experiments, can cause a transmission efficacy ranging from 0% to nearly 100%. Furthermore the larval and pupal mortality of infected hosts determines the survival of the pathogens and the growth of the host population.

**Contributed paper. Sunday, 3:00**

**Virulence and development of Johennea locustae in two locust species: Locusta migratoria and Schistocerca gregaria**

**Nuvva K. Manania**1, Larry J. Vaughan1,2, Ellie O. Osiri1 and Elizabeth O. Ouna1

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The microsporidium *Johennea locustae* was first described by Lange and co-workers in 1996 from the Malagasy migratory locust, *Locusta migratoria capito*. Microsporidium of the genus *Nosema* isolated from grasshoppers and locusts has been tested in field conditions with various results. We carried out an investigation of the susceptibility of *L. migratoria* and the desert locust, *Schistocerca gregaria*, to infection by *J. locustae* and its effects on fecundity and feeding. Both sides of wheat seedlings or spinach leaf discs (3-cm diameter) were sprayed with 10 ml suspensions of *J. locustae* produced from live host insects. Second-instars nymphs of both *S. gregaria* and *L. migratoria* were susceptible to *J. locustae* infection at the three exposure concentrations used (104, 105 and 106 spores ml−1). Mortality varied between 80 and 100% 32 days after treatment. There were no significant differences in morality between concentrations in both species. The LT50 values varied between 18.8-20.8 days with *L. migratoria* and between 21.8-24.9 days with *S. gregaria*. Spores of *J. locustae*
remained virulent to both *L. migratoria* and *S. gregaria* after three passages through *L. migratoria*. On the other hand, spores of *J. locustae* produced from *S. gregaria* hosts were only virulent against *S. gregaria*. The pathogen failed to complete its developmental cycle in *S. gregaria* after the first passage. For *S. gregaria* exposed to concentrations of 10^6, 10^5, or 10^4 spores ml⁻¹, there was a decrease in dry weight of food eaten at 10^6 spores ml⁻¹, but not at lower concentrations. Infected female *S. gregaria* nymphs did not survive long as adults to reproduce. Female *L. migratoria* surviving infection by *J. locustae* as nymphs laid significantly fewer pods than untreated controls at the three concentrations of 10^6, 10^5 and 10^4 spores ml⁻¹. The number of eggs per female was also significantly lower in treated lots than in the controls. However, there was no significant difference in egg viability between the different treatments.

Observations in natural populations showed that invading parasites spread quickly. In contrast to the observed impact of this parasite on host fitness, *O. bayeri* does not reduce host population density under semi-natural conditions.

**Symposium. Sunday, 5:20**

*Population and community level effects of microsporidia in trout stream food webs*

Steven L. Kohley¹ and Michael J. Wiley²

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Parasites and pathogens may have keystone effects in a community if they strongly affect the dynamics of other, strongly interacting species in the food web. We have observed such effects at large temporal and spatial scales in Michigan (USA) trout streams. In these systems, the herbivorous caddisfly *Glossosoma nigror* (Trichoptera: *Glossosomatidae*) is a particularly strong interactor, because larvae maintain the biomass (and productivity) of attached algae at very low levels throughout the year, which results in *Glossosoma* having strong competitive effects on most other primary consumers. *Glossosoma* is also host to a highly host-specific microsporidian, *Cougourdella* sp. Recurrent outbreaks of *Cougourdella* have resulted in whole-stream reductions in *Glossosoma* population size by 1-2 orders of magnitude, and maintained *Glossosoma* density at low levels for years. Fortuitously, we observed such outbreaks in many streams that were already the subject of long-term monitoring, allowing community-level effects of the pathogen to be assessed at large scales. Pathogen-induced reductions in *Glossosoma* abundance resulted in increased abundance of *Glossosoma*’s food resource (attached algae), and increased population sizes of most other algal consumers, including both grazers and filter-feeders. Several algal grazers (primarily other caddisfly species) that had been extremely rare or absent increased markedly following *Glossosoma*’s decline, indicating that they had been competitively excluded from these systems. *Cougourdella*’s effects in the community extended to higher trophic levels. Because pathogen-induced reduction in *Glossosoma* results in increased algal productivity and increased abundances of relatively vulnerable prey, presence of the pathogen should facilitate top-level carnivores and perhaps intermediate-level carnivores as well. Populations of predaceous caddisflies (*Rhynchocephila*) and stoneflies (*Paragnetina, Isoperla*) have increased over 2-fold following the collapse of *Glossosoma* populations in several streams, indicating that their populations are strongly resource-limited. Thus a host-specific pathogen, *Cougourdella*, initiates a trophic cascade in these systems by strongly affecting the abundance of a dominant competitor, *Glossosoma*. Pathogen-induced reduction in *Glossosoma* population size results in increased abundance and production of attached algae, and release from competition for other primary consumers. In many systems, predaceous invertebrates have responded to the increased abundance of relatively vulnerable primary consumers with increased population sizes, suggesting that their populations are also strongly food-limited.

**Symposium. Sunday, 5:50**

*Evolutionary strategies and adaptations for survival among mosquito-parasitic microsporidia and their intermediate copepod hosts*

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The natural epizootiology, transmission dynamics, and survival strategies employed by two mosquito-parasitic microsporidia that utilize copepods as intermediate hosts are examined in relation to the biological attributes of their respective hosts and the environments in which they inhabit. *Amblyospora connecticus*, a parasite of Ochlerotatus cantator and Ae. tracycheilopterus, is found in an unstable salt marsh environment that is subject to periodic flooding and drying. It’s hosts have distinct non-overlapping generations. *A. connecticus* exhibits a well-defined seasonal transmission cycle that...
relies heavily on maternal-mediated transovarial transmission by female *Oc. cantator* from June. through Sept., and horizontal transmission via the c moth host from Mar. to May (c moths to c moth) and Oct. to Dec. (c moth to c moth). It’s survival strategies include: low pathogenicity and high tissue specificity that allow for transdental transmission of horizontally acquired infections and maximum spore production, reliance on living hosts throughout most of its life cycle with over wintering in the c moth, polymorphic development that is well synchronized with host physiology, and production and dissemination of infectious spores that are coincident with the seasonal occurrence of susceptible stages each host. *Hyalina cysta chapmani*, a parasite of *Callosoma melanura* and *Orthoryctodes modestus* is found in a comparatively stable, forested, subterranean habitat is inundated with water throughout the year. C moths are omnipresent and *Cs. melanura* has overlapping broods. *H. chapmani* is maintained in a continuous cycle of horizontal transmission between each host from June. through Nov. but lacks a developmental sequence leading to transovarial transmission in the c moth host. It similarly relies on living hosts for most of its life cycle and over winters in diapausing c moth larvae and c moths. Transdental transmission does not occur and there is no polymorphic development in the c moth host. The spatial and temporal overlap of both c moth and c moth hosts during the summer and fall affords abundant opportunity for continuous horizontal transmission and increases the likelihood that *H. chapmani* will find a target host thus negating the need for a transovarial route. It is hypothesized that natural selection has favored the production of meiospores in female host mosquitoes rather than congenital transfer of infection to progeny via ovarian infection as a strategy for achieving greater transmission success. Analysis of the molecular phylogeny data suggest that transovarial transmission and the developmental sequence leading to ovarian infection may have been secondarily lost in *H. chapmani*, as they occur in all other closely related genera.

### VIRUSES – 1

**Contributed paper. Sunday, 4:20-6:20.**

**Isolation and characterization of baculoviruses from greenhouse populations of *Trichoplusia ni***

**Martin Erlandson**, Sarah Newhouse, Alida Janmaat, Keith Moore, Judith Myers, and David Theilmann

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The cabbage looper, *Trichoplusia ni*, is becoming an increasingly significant pest problem for greenhouse vegetable production in Canada. Control options are limited for producers who wish to maintain established biological control programs for other pests. To date, *Bacillus thuringiensis* (Bt) has been the agent of choice for *T. ni* control in greenhouses; however, indications of the development of resistance to Bt in c moth populations has been noted. Cabbage looper populations in greenhouses from the Fraser Valley of British Columbia were sampled and the larvae tested for sensitivity to Bt (Bt var kurstaki) and screened for the presence of baculoviruses. The susceptibility to Bt was determined by comparison of LC50 (IU/ml of diet) in laboratory assays of F1 larvae. All greenhouse populations were significantly less susceptible to Bt than a laboratory culture and to Dec. (mosquito to c moth). It’s survival strategies include: low pathogenicity and high tissue specificity that allow for transdental transmission of horizontally acquired infections and maximum spore production, reliance on living hosts throughout most of its life cycle with over wintering in the c moth, polymorphic development that is well synchronized with host physiology, and production and dissemination of infectious spores that are coincident with the seasonal occurrence of susceptible stages each host. *Hyalina cysta chapmani*, a parasite of *Callosoma melanura* and *Orthoryctodes modestus* is found in a comparatively stable, forested, subterranean habitat is inundated with water throughout the year. C moths are omnipresent and *Cs. melanura* has overlapping broods. *H. chapmani* is maintained in a continuous cycle of horizontal transmission between each host from June. through Nov. but lacks a developmental sequence leading to transovarial transmission in the c moth host. It similarly relies on living hosts for most of its life cycle and over winters in diapausing c moth larvae and c moths. Transdental transmission does not occur and there is no polymorphic development in the c moth host. The spatial and temporal overlap of both c moth and c moth hosts during the summer and fall affords abundant opportunity for continuous horizontal transmission and increases the likelihood that *H. chapmani* will find a target host thus negating the need for a transovarial route. It is hypothesized that natural selection has favored the production of meiospores in female host mosquitoes rather than congenital transfer of infection to progeny via ovarian infection as a strategy for achieving greater transmission success. Analysis of the molecular phylogeny data suggest that transovarial transmission and the developmental sequence leading to ovarian infection may have been secondarily lost in *H. chapmani*, as they occur in all other closely related genera.

**Contribution paper. Sunday, 4:35.**

**Genotypic and phenotypic variation of *Spodoptera exempta* nucleopolyhedrovirus**

**Libby Redman** and Jenny Cory

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The nucleopolyhedrovirus of the African armyworm, *Spodoptera exempta* (SpexNPV) is being developed as a biological control agent of this notorious pest. Although it is a highly virulent natural mortality agent and is ubiquitous in outbreak populations very little is known about its ecology and diversity. In order to characterize its diversity in natural populations individual infected larvae were collected in outbreaks from Northern Tanzania in 2001 and 2002 and subjected to restriction endonuclease (REN) profiling. A large level of variation was found within a small ecological scale with 15 genetically distinct isolates being identified within the same field. Within isolate variation was also investigated. Through the laborious method of vitro cloning a total of 10 viral clones have been isolated thus far. Ecologically and evolutionarily it is only important if these genetic differences though translate into phenotype. Standard bioassays were used to assess individual components of virus fitness such as mortality, speed of kill and yield. Differences were found for all three components. Genetic and biological comparisons have also been made between horizontally and vertically transmitted isolates.

**Field and safety assessment of genetically modified *Helicoverpa armigera* nucleopolyhedrovirus as a commercial insecticide**

**X. Sun, H. Wang, X. Sun, X. Chen, W. van der Werf, J.M. Vlak and Z. Hu**

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A baculovirus, *Helicoverpa armigera* nucleopolyhedrovirus (HaSNPV), has been developed as a commercial biopesticide to control cotton bollworm in China. To improve its insecticidal properties, the virus has been genetically modified either by deletion of the ecysteoyd UDP-glucosyltransferase (egt) gene from the viral genome (HaSNPV egt-minus) or by insertion of an insect-specific scorpion toxin (AaIT) gene replacing the egt gene. In laboratory bioassays, the speed of action of these recombinants was significantly higher than that of wild-type HaSNPV. The HaSNPV p6.9 promoter was the best to drive the expression of AaIT. To produce the recombinant HaSNPVs in vivo, a combination of optimal virus dose and a specific larval stage was selected to compensate as much as possible for the yield reduction of recombinant HaSNPVs as compared to wild type HaSNPV. In 2001 and 2002, a total of 150 kg of genetically modified HASNPV was produced. Permission was received from the Chinese Ministry of Agriculture to use these recombinants on cotton in a limited, large-scale field experiment and endonuclease analysis of genomic DNA from each isolate indicated that the TnSNPV isolates all had identical REN profiles which were distinct from a TnSNPV isolate (TsSNPV-RJ) previously collected from cabbage looper populations from New York (R. Jacques, AFFC, London, ONT). Among the AcMNPV isolates there were three distinct REN patterns observed. A series of dose response bioassays were conducted in 2nd, 4th, and 5th instar *T. ni* larvae to compare the infectivity and virulence of selected field isolates with AcMNPV-C6 and TsSNPV-RJ isolates. There was no significant differences in LD50 values for TsSNPV and AcMNPV-like isolates in 2nd instar LD50 ranging from 5 to 10 PIB/larva and 4th instar LD50 ranging from 10 to 20 PIB/larva. However, LD50s were significantly different in 5th instar *T. ni* larvae ranging from ~2500 PIBs/larva for one of the TnSNPV-like isolates to ~100 PIBs/larva for one of the AcMNPV-like isolates. The potential of these isolates for control of *T. ni* larvae on greenhouse vegetable crops is being investigated using spray trials on caged single plants for a variety of host plant species including tomatoes.
under certain conditions. Totally about 15 ha of cotton were treated with these recombinant HaSNPVs in the field for 2-8 times at various doses per hectare. The recombinant virus, carrying the AaFT gene, provided significantly better control of cotton bolls against bollworm damage than wild-type HaSNPV or HaSNPV eg- minus. Over an entire season and with natural infestations, cotton lint yield in plots treated by this recombinant was also significantly higher than that in wild-type HaSNPV-treated cotton plots. The pathogenicity against non-target animals (bee, silkworm, bird, fish and rat) and allergic responses in Guinea pig were investigated to identify potential ecological and health risks when using these genetically modified viruses as commercial insecticides. Furthermore their impact on nontarget parasitoids and predators, and their spread and persistence in the environment were monitored in field as well. No other effects, beyond what was observed for wild-type HaSNPV, were found. Acknowledgements: The authors appreciated the support from the 863 projects (010-06-10-01, 2001AA214031 and 2001AA212301), NSFC projects (30025083 and 39980001) and a joined grant from the Chinese Academy of Sciences and The Royal Netherlands Academy of Sciences (01CDIP023).

Control of false codling moth on citrus with a South African isolate of Cryptophlebia leucotreta granulovirus (CrleGV-SA)

Sean D. Moore1,2, Garth I. Richards1, Peter R. Stephen2, Bruce A. Tate1 and Donald A. Hendry2

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False codling moth, Cryptophlebia leucotreta (Meyrick) (Lepidoptera: Olethreutidae), is a fruit pest of citrus, macadamias, stone fruits, avocados and litchis, in southern Africa. Chemical control of C. leucotreta is problematic for a number of reasons. Recently, a novel isolate of the Cryptophlebia leucotreta granulovirus (CrleGV-SA) was described by restriction endonuclease analysis. In surface dose bioassays on artificial diet, LC50 and LC90 values with neonate larvae were estimated to be 4.095 x 10^7 OBs/ml and 9.118 x 10^9 OBs/ml respectively. LT50 and LT90 values with neonate larvae were estimated to be 4 days 22 h and 7 days 8 h, respectively. Detached fruit (navel orange) bioassays with neonate larvae indicated that virus concentrations that are likely to be effective in the field range from 1.08 x 10^10 to 3.819 x 10^10 OBs/ml. In surface dose bioassays with fifth instar larvae LC50 and LC90 values were estimated to be 2.678 x 10^10 OBs/ml and 9.118 x 10^10 OBs/ml respectively. LT50 and LT90 values were estimated to be 7 days 17 h and 9 days 8 h, respectively. These values are relevant for in vivo mass production of CrleGV-SA. In four field trials, unformulated crude CrleGV-SA consistently reduced C. leucotreta larvae infestation by around 60% for between five and nine weeks. CrleGV-SA formulated with molasses and a wetter reduced C. leucotreta infestation by around 80% over a nine-week period in two trials. These results were consistently better than those achieved with the insect growth regulator, triflumuron. Reasons for these impressive results and prospects for future use of CrleGV-SA in integrated pest management are discussed.

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Contributed paper. Sunday, 5:35

Advances towards improving the insecticidal properties of AgMNPV

V. Romanowski1,2, E.I. Araúa1, C.B. McCarthy1, M.E. Biedma1, A. Scioce-Cap2, A.V. Goldberg2, P.D. Ghiringhelli3, F.J. Pinedo4, F. Moscardi5, B. M. Ribeiro6

1IBMM, Fac. Ciencias Exactas, Univ. Nacional de La Plata; 2IMYZA, INTA Castelar, 3Univ. Nacional de Quilmes; Argentina; 4Dep. de Biología Celular, Univ. de Brasilia; 5 CNPSo-EMBRAPA, Londrina, Brazil

Anticarsia gemmatalis is a key pest of soybean in Brazil, Argentina, and other countries. AgMNPV is today the most widely used baculovirus pesticide, as more than two million hectares are treated annually. However, a number of problems prevent the expansion of the use of the virus to the ca. 20 million hectares of soybean cultivated in South America. A major drawback is the slow speed of kill of wt AgMNPV, which becomes extremely important in areas with lower temperatures (20°C). In order to address this problem we have recently developed a system for the genetic modification of AgMNPV. To expand the number of alternative genetic modifications we introduced two unique sites for the intron-encoded Ippol endonuclease to linearize the viral DNA used in cotransfections, which greatly reduced the background of non recombinant progeny. The insertion of the insect-specific neurotoxin gene isolated from the mite Pyemotes tritici (TxP-1) yielded a rAgMNPV that paralysed the host larvae within two days after treatment. On the other hand, the disruption of the egt gene eliminated the viral enzyme that inactivates edesynone, thus accelerating the moulting and the cessation of feeding. The egt(−) rAgMNPV killed the larvae 1-2.8 days faster than the wt virus (mean reduction of LT50 across virus concentrations: 2.2 days) and exhibited a higher virulence (LC50 3.9-fold lower than wt). Both rAgMNPVs significantly reduced the damage caused by the pest. Additionally, strategies of host range expansion in order to control simultaneous lepidopteran pests would certainly increase the appeal of AgMNPV to soybean growers. Controlled field experiments will address the applicability of these and other genetically improved AgMNPVs in large scale pro-
grams. The traceability of the recombinants will be facilitated by the insertion of reporter genes.

Contributed paper. Sunday, 5:50

Comparing transmission between LdNPV strains: “liquefying” vs. “non-liquefying”

Vincent D’Amico1, John Podgwaite1, Ralph Webb2, Kevin Thorpe2, Roger Fuester2, Mike Valenti3, Randy Pfeiffer5, Phil Taylor7, and Jim Slavicek3

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Dover, DE 19901-5515 The gypsy moth (Lymantria dispar) nucleopolyhedrosis virus (LdNPV) is an occluded double-stranded DNA virus which causes epizootics in naturally occurring gypsy moth populations wherever they occur. Gypsy moths become infected by consuming the contaminated surface of the egg at hatch, or eating contaminated foliage. All LdNPV infections occur per os. The wild-type virus produces chitinase in the later stages of infection, which causes dead larvae to break open and spill the LdNPV filled interior onto the surface of leaves. This is thought to be vital to the horizontal transmission of disease to later instar larvae. However, it is also possible that virus contained within a cadaver would be better protected from UV light, and would be released over a longer period of time. To explore this question, we isolated and produced a strain of LdNPV which does not cause liquefaction in the dead host. This strain of LdNPV was used to infect early instar larvae. The larvae were confined on outdoor foliage in mesh bags until death, and later instars were used to assay this foliage for one week under conditions closely resembling those in the field. The same methodology was simultaneously used to assay the wild-type virus for comparison.

Strains of Plutella xylostella (diamondback moth) isolated from the Serdang region of Malaysia have been shown to be resistant to the Cry1Ac toxin produced by the bacterium Bacillus thuringiensis. The biochemical basis of the mechanism of resistance of this population was investigated and we will present the results of comparative biochemical studies, including protease assays, in vitro and in vivo processing of the protoxin and binding assays. Finally we will present bioassay data showing how an engineered variant of Cry1Ac is over 600 times more toxic to the resistant population than wild-type Cry1Ac. Unexpectedly this variant was also considerably more active than wild-type toxin against a susceptible population of Plutella.

Contributed paper. Sunday, 4:50

Resistance to Bacillus thuringiensis endotoxins in the European corn borer (Lepidoptera: Crambidae)

Huarong Li1, Joel Gonzalez-Cabrera1, Brenda Oppert1, Juan Férre1, Randall A. Higgins1, Lawrent L. Buschman1, Kun Yan Zhu1, and Fangneng Huang1

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Bacillus thuringiensis (Bt)-based biotechnology is threatened by the development of Bt-resistant pests. Understanding physiological changes in Bt-resistant insects will help to design more effective resistance management strategies for sustaining Bt-based biotechnology. Research has characterized two types of Bt resistance mechanisms in insects: reduced toxin binding to receptors in the brush border membrane (BBM) of insect midguts, and decreased activation of Bt protoxin by reduced activities of midgut proteases. Resistance mechanisms in a Dipel-selected European corn borer (ECB) strain were studied by comparing Cry toxin binding to BBM vesicles and larval gut protease activities of resistant and susceptible larvae. Binding of Cry1Ab and Cry1Ac was compared in resistant and susceptible ECB larvae using three different methods. Ligand blot assays demonstrated no apparent differences in the number of toxin binding proteins in BBM vesicles of resistant and susceptible larvae, and the relative binding intensities of either Cry1Ab or Cry1Ac were similar. Surface plasmon resonance assays demonstrated that the specific binding of Cry1Ab to BBM vesicles from resistant and susceptible larvae was also similar. Radiolabeled toxin binding analysis indicated no significant differences in the binding affinity of either Cry1Ab or Cry1Ac between resistant and susceptible ECB larvae, and demonstrated that Cry1Ab and Cry1Ac share binding sites as well. Overall, the binding analyses suggest that resistance to Cry1Ab and Cry1Ac in this strain of ECB is not associated with differences in toxin binding. However, the activity of soluble trypsin-like proteases in resistant ECB larvae was reduced 56%, and Cry1Ab protoxin activation by proteinase extracts from the resistant larval gut was reduced 32% compared to susceptible larvae. Therefore, reduced protoxin activation may contribute to resistance in this strain of ECB, although other mechanisms, such as impaired pore formation, are possible. These results suggest that transgenic Bt plants expressing full-length protoxin even semi-truncated toxin at low to moderate levels may increase the potential of resistance development in target pests.

Contributed paper. Sunday, 5:05

The effect of genetically modified insect-resistant Brassica plants on non-target invertebrates

Rachael E. Collier1, Rosemary H. Collier1 and Chris C. Payne2

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Genes from Bacillus thuringiensis (Bt) have been introduced into more plant species than any other insecticidal genes. The aim of this project is to determine how feeding on Bt-transformed Brassica plants affects non-target pest insects and their natural enemies. By using a multi-disciplinary approach, involving biochemistry, molecular biology and insect biology, it should be possible to obtain a thorough understanding of tri-trophic interactions between transgenic

Contributed papers. Sunday, 4:20

Inheritance of resistance to Bacillus thuringiensis kurstaki in Trichoplusia ni

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Resistance to Bacillus thuringiensis kurstaki in Trichoplusia ni populations has become an increasing problem in commercial vegetable greenhouses in British Columbia, Canada. One moderately and one highly resistant greenhouse T. ni population, 23-fold and 86-fold more resistant than a reference laboratory colony respectively, were established in the laboratory for genetic analysis. To examine the inheritance of resistance, F1 progeny of reciprocal crosses of resistant adults and adults of a susceptible laboratory colony were assayed for resistance to Btk (Dipel, Abbott). Backcrosses of the F1 progeny to the parental populations were performed to determine if the resistance trait is monogenic. Analysis of the reciprocal crosses and backcrosses of the moderately resistant colony indicate that resistance is inherited as an autosomally recessive trait and controlled by more than one locus. For both the moderately and highly resistant colonies, resistance rapidly decreased in the absence of selection indicating that resistance is associated with a fitness cost.

Contributed paper. Sunday, 4:35

Understanding and overcoming resistance of Plutella xylostella to Bacillus thuringiensis Cry1Ac toxin

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1School of Biological Sciences, Univ. of Sussex, UK; 2Dept. of Biological Sciences, Imperial College, UK

The Cry1Ac toxin produced by the bacterium Bacillus thuringiensis. The biochemical basis of the mechanism of resistance of this population was investigated and we will present the results of comparative biochemical studies, including protease assays, in vitro and in vivo processing of the protoxin and binding assays. Finally we will present bioassay data showing how an engineered variant of Cry1Ac is over 600 times more toxic to the resistant population than wild-type Cry1Ac. Unexpectedly this variant was also considerably more active than wild-type toxin against a susceptible population of Plutella.
plant, pest insect and parasitoid or predator. The cabbage root fly (Delia radicum) and its natural enemies are being used as a model system. Cabbage root flies were reared on transgenic or non-transgenic (F1 hybrid) Brassica plants for three generations, to determine whether feeding on Bt-expressing transgenic plants affected their development. Cabbage root flies reared on transgenic plants were heavier (mean pupal weight) than those reared on the non-transgenic (F1 hybrid) plants. Analyses showed that the relationship between pupal weight and the amount of Bt expressed in individual plants was non-linear. Further studies to determine whether the differential pupal weights were due to the presence of Bt have been conducted.

**STU** Contributed paper. Sunday, 5:20

**Studying Cry1C-resistance mechanisms by using Sf9 cells**

Dror Avisar, Baruch Sneh, Nor Chejanovsky1 and Aviah Zilberstein

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Spodoptera frugiperda cell line Sf9 is highly sensitive to Bacillus thuringiensis d-endotoxin Cry1C. We have been using Sf9 as a model system for unraveling Cry1C mode of action and possible receptors. We have identified, isolated and established Cry1C tolerant Sf9 cell lines (rSf9) using a random stable gene silencing approach. The resulting rSf9 cell lines are resistant to low concentrations of Cry1C up to 250ng/ml. When exposed to higher concentrations of Cry1C, the rSf9 cell lines are eventually affected, but at a much lower rate compared to the wild type Sf9 cell line and can be easily rescued from the toxin treatment.

Another approach, based on microscopic observations of Cry1C treated Sf9 cells, has revealed that tolerance to Cry1C is a cell cycle phase dependent. Metaphase and early G1 Sf9 cells are totally resistant to Cry1C. Upon exposure to Cry1C, M-phase cells could complete the cycle, whereas the rest were killed by the toxin within 120 min. Treatment with nocodazole, a M-phase cell-arresting agent, synchronized the cells in M-phase turning 90% of the cells resistant to Cry1C. The M-phase arrested Sf9 cells (mSf9) regained Cry1C sensitivity after washing out the arresting agent. In the presence of Cry1C, the nocodazole released cells gradually died during other phases of the consecutive cell cycle, indicating that the transient Cry1C-insensitivity only exists during the M-phase.

A cDNA library subtraction strategy is currently being used to isolate genes that are involved in dictating Cry1C tolerance in the rSf9 cell line and during M-phase.

Contributed paper. Sunday, 5:35

**Selection with Bacillus sphaericus Plus Cyt1Aa from Bacillus thuringiensis subsp. israelensis: effect on Bacillus sphaericus resistance in mosquitoes**

Margaret C. Wirth1, Joshua A. Iannino1, Brian A. Federici1,2, and William E. Walton1

Dept. of Entomology1 & Interdepartmental Graduate Program in Genetics2, Univ. of California, Riverside, California 92521, USA

There is a substantial difference in the risk for insecticide resistance in mosquitoes that are treated with Bacillus sphaericus compared to mosquitoes treated with Bacillus thuringiensis subsp. israelensis. Resistance can be rapidly selected with B. sphaericus but not with B. t. subsp. israelensis and this difference in risk is related to both the toxin complexity and toxin interactions that naturally occur in B. t. subsp. israelensis. B. t. subsp. israelensis expresses a mixture of 4 major proteins, Cry4A, Cry4B, Cry11A, and Cyt1Aa. The contribution of the Cyt1Aa toxin is intriguing because it is extremely difficult to induce resistance in the presence of Cyt1Aa, whereas resistance can be induced against B. t. subsp. israelensis in the absence of Cyt1Aa. Cyt1Aa is known to interact synergistically with the Cry toxins in B. t. subsp. israelensis and this synergism was found to suppress resistance. Further experiments demonstrated that selection with a mixture of Cyt11A + Cyt1Aa (3:1) delayed the onset of resistance and ultimately conferred a lower level of resistance than selection with Cyt11A alone. Cyt1Aa has also been demonstrated to interact synergistically with B. sphaericus against B. sphaericus-resistant mosquitoes and, importantly, to suppress B. sphaericus resistance, which suggests that it may help retard the evolution of resistance to this material. To test this hypothesis a synthetic laboratory population of Culex quinquefasciatus, consisting of a mixture of susceptible and Bacillus sphaericus-resistant mosquitoes, was selected for 20 generations with B. sphaericus 2362 or a mixture of B. sphaericus + Cyt1Aa (3:1) and changes in susceptibility were monitored. Similar to prior selections using B.t. subsp. israelensis, the mixture of B. sphaericus and Cyt1Aa retained high activity against both selected lines and induced lower resistance levels than selection with B. sphaericus.

Contributed paper. Sunday, 5:50

**Phylogenetic diversity within Bacillus thuringiensis and Bacillus cereus isolates: Only one group has pathogenic or toxigenic properties in vertebrates**

Paul J. Jackson, Karen K. Hill, Lawrence O. Ticknor, Charles H. Helma and Richard T. Okinaka

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Amplified Fragment Length Polymorphism (AFLP) and Single Nucleotide Polymorphism (SNP) analyses of multiple genes from a large collection of Bacillus isolates demonstrate that B. cereus and B. thuringiensis are highly polymorphic species. Phylogenetic and principal component analyses identify three main clusters that each contains B. cereus and B. thuringiensis isolates interspersed with one another. Many B. thuringiensis isolates are more closely related to B. cereus isolates than to other B. thuringiensis isolates and the converse is also true. Cluster one contains most of the B. thuringiensis isolates that are currently used in biopesticide preparations. Cluster two, which also contains all known B. anthracis isolates on a single branch of the phylogenetic tree, is clearly distinct from clusters 1 and 3 and contains the majority of the known pathogenic and toxigenic B. cereus and B. thuringiensis isolates. Several of these latter isolates have been associated with severe infections in animals or humans while others are responsible for food-borne illnesses. All of the B. cereus and B. thuringiensis isolates that map to this cluster can be distinguished from B. anthracis. Cluster three contains primarily B. cereus environmental isolates interspersed with B. thuringiensis isolates. The implications of these results in developing strategies for the use or release of different B. thuringiensis and B. cereus isolates will be discussed.

**CONTRIBUTED PAPERS. Sunday, 4:20-6:20.**

**FUNGI – 1**

Contributed paper. Sunday, 4:20

**Isolate selection and formulation of Beauveria bassiana for controlling tarnished plant bug, Lygus lineolaris (Heteroptera: Miridae) in wild host plants**

Jarrod E. Leland1 and Robert W. Behle2

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The tarnished plant bug, Lygus lineolaris, has become an increasingly important cotton pest due to changes in control practices for lepidopterans (i.e. introduction of Bt cotton and more specific insecticides) and following boll weevil eradication. High density populations of L. lineolaris develop in wild host plant areas, which are restricted in intensively agricultural regions, before moving into cotton. Previous work has evaluated controlling wild host plants for the area-wide management of L. lineolaris. Microbial control agents may provide another option for controlling these populations with reduced environmental impacts. Entomopathogenic fungi offer the greatest potential of the microbial control candidates for controlling L. lineolaris due to their contact mode of action and L. lineolaris' piercing sucking mouthparts. An ideal mycoinsecticide would have low impact on non-target species, high virulence to L. lineolaris, and
high environmental stability. New isolates of *Beauveria bassiana* have been obtained from indigenous *L. lineolaris* populations that have greater virulence to *L. lineolaris* than commercially-available isolates. The specificity of these isolates are being evaluated using representative beneficial insects including: ladybugs (*Hippodamia convergens*), pirate bugs (*Orius insidiosus*), lacewings (*Chrysopa carnea*), praying mantids (*Tenderea aridifolia sinensis*), and parasitic wasps (*Anaphes isolex*). New formulation strategies are being evaluated for *B. bassiana* involving the use of lignin-coated spores, which greatly improve spore survival following exposure to solar radiation.

**Contributed paper. Sunday, 4:35**

**Impact of Beauveria bassiana on Western tarnished plant bug**

Michael R. McGuire

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The Western tarnished plant bug, *Lygus hesperus*, is a pest of many crops including strawberries, seed alfalfa and cotton. Damage to California cotton alone can reach hundreds of thousands of dollars annually. Currently, no specific controls exist and application of broad spectrum pesticides may eliminate natural enemies and flare secondary pests. Published laboratory studies suggested that *Lygus* species are susceptible to *Beauveria bassiana* but field tests with commercial isolates did not result in significant population reductions. In a search for natural enemies of *L. hesperus* in the San Joaquin Valley of California, adults and nymphs were collected from alfalfa fields and roadside vegetation and held individually in the laboratory. In virtually all fields surveyed and at all times of the year, *B. bassiana* was found infecting *L. hesperus*; in some samples, infection levels exceeded 50%. Intensive weekly sampling of several alfalfa fields over a two year period did not reveal a clear relationship between population size and percentage infection but *B. bassiana* was observed during climatic conditions normally not associated with the fungus. Laboratory studies demonstrated that isolates collected from the SJV could grow at temperatures exceeding 32E C suggesting adaptation to local climatic conditions. In addition, laboratory bioassays demonstrated that *B. bassiana* isolated from *L. hesperus* had much higher activity against *L. hesperus* than a commercial strain of *B. bassiana*. Experiments continue on strain characterization and the behavior of inoculated insects.

**Contributed paper. Sunday, 4:50**

**Evaluation of bee pollinators as vectors of Beauveria bassiana for control of the tarnished plant bug and western flower thrips on greenhouse peppers**

M.S. Al-mazra’awi1, J.L. Shipp2, A.B. Broadbent3, and P.G. Kevan1

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The ability of pollinators such as honey bees and bumble bees to vector biological control agents has been demonstrated. The use of this capacity to vector entomopathogenic fungi is a novel application to greenhouse peppers. Experiments were conducted at the Greenhouse and Processing Crop Research Centre in 2003 to investigate the ability of bumble bees to transfer inocula of *Beauveria bassiana* [BotaniGard WP mixed with corn flour] to greenhouse pepper flowers for subsequent control of the tarnished plant bug, *Lygus lineolaris*, and the western flower thrips, *Frankliniella occidentalis*. Commercial colonies of bumble bees, *Bombus impatiens*, with inoculum dispensers at hive exits, were allowed to forage on pepper plants inside large screened enclosures within 2 greenhouse compartments. Samples of bees, pepper flowers and both pest species from plants were collected throughout the trials to quantify the presence and infection levels of *B. bassiana*. Preliminary results will be presented. Application of pollinator-vectored technology not only contributes to pest management but also improves fruit yield and quality by improving pollination and fruit set.

**Contributed paper. Sunday, 5:05**

**The effect of changing application rate, volume, and interval on acquisition of Beauveria bassiana conidia by Western flower thrips and resulting control in garden impatiens**

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The Western flower thrips (WFT), *Frankliniella occidentalis*, causes significant economic losses to various greenhouse crops via feeding damage and virus transmission. Its ability to rapidly develop resistance to insecticides and its high level of susceptibility to *Beauveria bassiana* (strain GHA) in the laboratory (LD50’s for adult female and second instar nymphs of 5 and 47 conidia per insect, respectively) make this pest a promising candidate for microbial control in greenhouse crops.

A crop of garden impatiens infested with WFT was sprayed with 1lb/100gal/acre of the *Beauveria bassiana*-based BotaniGard 22WP once a week for 3 weeks. This protocol did not result in adequate control of the population after three applications. In an attempt to improve efficacy of the BotaniGard 22WP product, a series of independent experiments that varied the spray parameters, application interval, application rate, and application volume were conducted.

Crops of garden impatients infested with WFT were treated with 1lb/100gal/acre BotaniGard 22WP at spray intervals of 3d, 5d, and 7d, at application rates of 1, 2, 4 and 6lbs/100gal/10,000 square feet, and at volumes of 25, 50 and 100gal/10,000 square feet (1lb/1000 square feet). Applications were made weekly for three weeks. Samples of pollen bearing impatients flowers were taken twice weekly to estimate thrips population density, and adult female and second instar thrips were collected 24h post-inoculation for determination of dose (conidia/insect).

There was a slight difference in the rate of population growth in the application interval and application volume bioassays; however, varying spray parameters did not lead to significant levels of control in any of the experiments. The conidia per insect increased linearly with increasing application interval and application volume. The number of conidia per insect was not affected by application rate.

**Contributed paper. Sunday, 5:20**

**Evaluation of two microbial pesticides for integrated thrips control in glasshouse chrysanthemums**

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*Frankliniella occidentalis*, the Western Flower thrips, is a major pest species in Chrysanthemum. In the Netherlands, lack of selective and effective pesticides for its control is one of the main causes for failure of introducing integrated pest management (IPM) programmes. The aim of our research in Chrysanthemum, funded by the Dutch Product Board for Horticulture (Productschap Tuinbouw), is to evaluate experimental and existing microbial, botanical and chemical pesticides and natural enemies, in order to develop a feasible IPM programme for this crop.

In 2002 a glasshouse experiment with Chrysanthemum was carried out with the microbial pesticides NemasySF (*Steinernema feltiae*, Becker Underwood) and Mycotal/Addit (*Verticillium lecanii*, Koppert BV). These agents were tested with and without the presence of the thrips predator *Amblyseius cucumeris* (Thripex-plus, Koppert BV, 1 sachet/m2, applied once). NemasySF, Mycotal and water treatments were applied weekly in 8 plots (2.5 m2 each) with and 8 plots without predatory mites, with a total of 9 applications.

After 5 weekly applications of NemasySF (107 nematodes/m2 + spreader) 74% less thrips were found compared to plots with weekly water applications. Also at harvest, after 9 weekly applications of NemasySF, 74% less thrips were found. When at the same time predatory mites were present in the NemasySF plots, respectively 82% and 96% less thrips were found compared to the water treated plots. After 5 weekly applications of Mycotal (109 conidia/m2 with Addit) 38% less thrips were found compared to plots with weekly water applications. At harvest, after 9 weekly applications of NemasySF...
Mycotal, 14% less thrips (not significant) were found. When also predatory mites were present in the Mycotal plots, respectively 62% and 70% less thrips were found.

In this glasshouse trial weekly applications of the nematode product NemassyS® resulted in a highly considerable reduction of thrips in the Chrysanthemum crop. Weekly applications of the microbial pesticide Mycotal gave rise to a less substantial reduction of thrips. It was also shown that both products can be used in combination with the thrips predator A. cucumeris, causing a higher reduction of the thrips population.

**STU** Contributed paper. Sunday, 5:35

**Management of sucking pests with *Beauveria bassiana* in Australia**

Kristen Knight, David Holdom, and Caroline Hauxwell

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Australian cotton and grain growers are using biopesticides based on insect pathogens on a large-scale as part of an Integrated Pest Management (IPM) strategy for *Helicoverpa* species. Biopesticides are used to control pests while maintaining beneficial insect populations and avoiding creation of resistance to chemical insecticides.

As pest management practices change, sucking pests such as mirids (*Creontiades* sp.) and green vegetable bug (*Nezara viridula*) are emerging as a significant problem. There are currently no selective insecticides for sucking bugs, while application of broad-spectrum insecticides threatens the IPM strategy by destabilizing natural enemy populations and triggering outbreaks of *Helicoverpa* sp. The grains and cotton industries have thus supported our research into development and testing of biopesticides based on entomogenous fungi against sucking pests.

Initial field and laboratory assays have shown that an Australian *Beauveria bassiana* isolate EFD 36 is highly active against both mirids and *Nezara*. In bioassays against 1st instar *Nezara* nymphs, EFD 36 caused 80% mortality within three days at 1 x 10^8 spores/ml. Field trials against mirids gave control of nymphs (but not adults) equal to the chemical dimethoate at six days after treatment, and was twice as effective as the commercial *Beauveria* product Mycotal®. Comparison of oil and emulsifiable formulations suggests that application method (ULV or conventional) may have more impact on performance than formulation.

Season-long monitoring of pests has indicated that sucking pest populations build up in pulse crops through the early season. We are developing an IPM strategy based on microbial control of nymphs during early-season establishment, while maintaining beneficial insect populations.

**Efficacy of *Beauveria sp.* in the control of first instar larvae of the Andean Potato Weevil (*Premnotrypes suturicallus* Kuschel)**

Magnus Kühl,1 Stefan Vidal,2 Kerstin Jung,3 Dietrich Stephan4 and Aziz Lagnau5

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The Andean Potato Weevil (*Premnotrypes suturicallus*, APW) is a serious insect pest affecting potato production in the high Andes, causing up to 50% yield loss through tuber damage. The objective of this research project is to investigate the use of entomopathogenic fungi to control the first instar larvae before they enter the tuber. Potato tubers were placed at the bottoms of 18 cm high receptacles which were filled up with sterilized soil. In treatment 1 (T1), the larvae were directly infected with a *Beauveria sp.* solution (2 x 10^8 spores/ml); in treatment 2 (T2) the soil was infected (5 x 10^8 spores/g) of soil; treatments were replicated 6 times. Larvae were liberated on top of the soil. After 1 month, controls showed a mortality of 3%, compared to 22% in T1 and 12% in T2. Further observations of the surviving fourth instar larvae (several of which had already left the tuber and entered the soil) showed high infection rates in T2 (45%) (T1 4%, control 7%). Pathogenicity was again tested by infecting first instar larvae and placing them on tubers for 12 days. Mortality with isolate CIPCa1 was 40%, with CIPH40 was 45% and in the control was 12.5%. Each treatment was replicated 5 times. The reasons for the low susceptibility of the first instar larvae were tested by infecting larvae directly with *Beauveria sp.* As control, larvae were kept without tubers; in treatment 1 (T1) larvae were placed on tubers and in treatment 2 (T2) larvae were left to pass through the soil. After 0, 1 and 2 days, one group (G1) of each treatment was placed individually for 1.5 h on antibiotic agar and the other group (G2) was ground in TWEEN 80 (0.1%) and this solution was then applied to antibiotic agar. In the control, Colony forming units (CFU) of G2 was >100, 69 and 95 after 0, 1 and 2 days respectively, in T1 23 and 5 after 1 and 2 days and in T2 16 after 2 days. Results for G1 (CFU) were: Control 78, 76 and 81 after 0, 1 and 2 days; T1 8 and 7 after 1 and 2 days; and T2 2 after 2 days. The presence of conidia on the larvae is significantly reduced after passage through the soil or entering the tuber. These results indicate, that the first instar larvae are difficult to target with entomopathogens for the control of APW.

**Comparative virulence and host specificity of *Beauveria bassiana* isolates assayed against lepidopteran pests of vegetable crops**

S.P. Wraith1, M.E. Ramos1, J.E. Williams1, P.B. Avery2, S.T. Jaronski2, and J.D. Vandenberg3

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Approximately 40 isolates of the entomopathogenic fungus *Beauveria bassiana* were screened against second-instar larvae of diamondback moth (*Plutella xylostella*) (DBM), European corn borer (*Ostrinia nubilalis*) (ECB), corn earworm (*Helicoverpa zea*) (CEW), and fall armyworm (*Spodoptera frugiperda*) (FAW), and 30 of these isolates were tested against beet armyworm (*Spodoptera exigua*) (BAW). Highly virulent isolates identified in the screening assays were also tested against black cutworm (*Agrotis ipsilon*) (BCW), and the top isolate was also assayed against imported cabbage worm (*Pieris rapae*) (ICW) and cabbage looper (*Trichoplusia ni*) (CL). *B. bassiana* was pathogenic against all lepidopteran species tested, and numerous highly virulent isolates were identified. Corn earworm and beet armyworm were most susceptible to fungal infection, and fall armyworm was least susceptible. Limited testing suggested low susceptibility also of black cutworm and cabbage looper. A unique isolate (strain BB1200) exhibited virulence against all pest species greater than or equal to the most important commercial strain of *B. bassiana* currently registered in the U.S. (strain GHA). In assays in which larvae were topicaly sprayed and maintained on the treated substrate for 24 h at 100% relative humidity, 6-day (25°C) median lethal rates (LR50s) of this isolate against CEW, BAW, DBM, FAW, ICW, ECB, CL, and BCW were 4, 5, 7, 11, 12, 98, 125, and 273 conidia/mm², respectively. The respective LR50 of commercial strain GHA against these pest species were 9, 67, 97, 1213, 29, 1668, 541, and 3504 conidia/mm². Use of LR50 versus median lethal dose ratios (comparing LR50 of each isolate to a “standard” strain) generated similar rankings of isolate virulence.

**Contributed paper. Sunday, 6:05**
SYMPOSIUM (Cross-Divisional). Monday, 8:00-10:00.
Is bigger always better? A comparison of industrial-scale vs. cottage industry-scale production of microbial pesticides

Symposium. Monday, 8:05
D. Moore2

Do we have it in the bag? - Production of Metarhizium anisopliae

L. Langewald1, N. E. Jenkins2, B. Ali3, M. Brüntrup4 and D. Moore2

1ITA, Cotonson, B.P. 08-0932, Benin; 2CABI Bioscience, Silwood Park, Ascot, SL5 7TA, UK; 3CABI Bioscience, Caribbean and Latin America Centre, Trinidad and Tobago; 4Freelance consultant, Stuttgart, Germany

The simple and well documented ‘low tech’ production of Metarhizium anisopliae on autoclaved rice in bags/bowls has proven to be a valuable and reliable method for small to large quantities of product for experimental and commercial use. Systems using such ‘labour intensive’ techniques vary from small-scale artisanal type systems employing nothing more technical that a pressure cooker, to relatively sophisticated facilities with a throughput of 100s tones colonized substrate/annum.

Bag-based systems have been criticised over poor quality, poor economics, poor worker safety, unethical use of valuable cereal products, lack of process control, low capacity, limited scale-up and lack of reliability. Whereas examples of production units exhibiting any one or all of these attributes certainly exist, in our experience there also exist a number of facilities for which none of the above apply. They may not even be labour intensive; bag-based systems can be supported by highly technical equipment, with resulting low labour input.

Industrial ‘high tech’ production of Metarhizium on the other hand is rather less well documented and is characterized by very few examples of successful use of automated fermentation process equipment from which reliable products are regularly produced. The majority, if not all these systems, were designed for the production of other fungi, such as Beauveria or Paecilomyces in mind. Metarhizium is notably more difficult to produce, often resulting in lower yields and being prone to contamination. These difficulties have a serious impact on production costs.

In this paper we present a detailed business plan for a high quality but ‘low tech’ bag based production system to be installed in a developing country and we describe ways how this technology can stepwise be redesigned into an industrial style system appropriate for developing countries with major differences in labour costs.

We suggest that the question is not whether ‘industrial or cottage-style industry’ is better, but what is it that defines an industrial system.

Symposium. Monday, 8:28

Entomopathogenic nematode production

David I. Shapiro-Ilan

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Entomopathogenic nematodes (genera Steinernema and Heterorhabditis) kill insects with the aid of symbiotic bacteria. The nematode-bacteria complex can be mass-produced for use as biopesticides through in vivo or in vitro methods (solid or liquid fermentation). The production technology that is most appropriate to a given system will vary depending on market demand, available technical expertise, and capital. In vivo production requires low technology, has low startup costs, and resulting nematodes quality is generally high, yet cost efficiency is generally deemed to be low. Liquid culture is deemed to have the greatest cost efficiency, but requires a high level of technical expertise, large capital outlay, and quality issues may ensue. Liquid culture may be improved through progress in media development, nematode recovery, and bioreactor design. In vitro solid culture, i.e., growing the nematodes and bacteria on crumbled polyurethane foam, offers an intermediate level of technology and costs. In vivo production and solid culture may be vastly improved through innovations in mechanization and streamlining. Potential approaches to increasing cost efficiency of in vivo production include, adoption of the recently developed “LOTEK” scalable system, use of alternate hosts, or application of nematode-infected host cadavers directly to the target site.

Symposium. Monday, 8:51

Production of biopesticides in developing countries: the roles of cottage industry, NGOs, state sector enterprises and private commercial producers in Asia

David Grzywa2, Uthai Ketunuti2 and Hilary Warburton1

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Baculoviruses offer environmentally acceptable crop protection technology. It is often claimed that an advantage of biopesticides such as NPV and fungi is that they can be produced in low technology systems appropriate for developing countries. However uptake and production of entomopathogenic viruses in developing countries is still very limited and identifying appropriate systems for biopesticide production and supply is still a key issue.

A survey of biopesticide promotion strategies in two Asian countries India and Thailand illustrates some of constraints that biopesticides face. In these countries production models adopted have ranged from capital-intensive industrial scale production in purpose built factories, to field based production by farmers themselves. Production has been established by commercial pesticide companies, national agricultural extension services, international research institutes, and non-governmental organisations focused on alleviating rural poverty. These different producers have had various degrees of success often crucially related to the quality of product they can maintain. While farmer and community based initiatives have been seen as an answer to making biopesticides available to poor farmers the quality control problem is a serious problem for this approach. Private commercial production can reach acceptable quality standards but poor quality products are still a problem where regulation is inadequate. The state sector can be an effective producer, where adequate resources and are made available, but state organisations do not always have the expertise and systems to effectively produce and market biopesticide products.

Farmer satisfaction with biopesticides can be surprisingly high where promotion has been to appropriate cropping systems, products are effective and pricing competitive. Little success is seen in promoting biopesticides in low value cereals and fibre crops In contrast in the higher value horticultural and fruit sector, where there are insecticide resistance and residue problems are key issues, biopesticides can have a real competitive advantage and some significant progress can be seen.

Symposium. Monday, 9:14

Commercializing mycoinsecticides: The U.S. Experience

Stefan T. Jaronski

USDA REE ARS NPARL, Sidney MT USA

(formerly Manager Biopesticide R&D, Mycotech Corp., Butte MT)

In the United States, biopesticide implementation is structured by the need to register any microbial with the Environmental Protection Agency and with individual states, some of whom can be more stringent than the federal agency. This process has significant time and money requirements approximately two years on average of data and its review by regulators, and close to $1MM in internal and external costs, in addition to normal operating costs. Framed against this structure is an economic model in which private companies are almost the only route for commercialization in the US; federal involvement through a state supported enterprise is statutorily minimal. The momentum of biopesticide development, therefore, has been largely with small companies. (Large, long established, agchemical companies have not been able to sustain a development effort where any has existed at all.) These small biopesticide enterprises subsist heavily on private venture capital, from investors whose ultimate goal is to make money in as little time as possible, and who may not understand the business. Meanwhile, American agriculture still operates in a largely chemical paradigm, one in which microbial agents...
may not easily fit. Thus, their successful development is often a challenge. Lastly, the biopesticide distribution system in the U.S. is expensive, which expense can add considerably to the cost of a microbial product. These aspects structure practical market sizes for a biopesticide, and, in turn, production capacity and efficiencies. Target crops have to be of sufficient size and nature so that low market penetration (in the face of chemical paradigms) still provides sufficient revenues to meet development (and survival) costs. Thus, some crops may have very low priority, and multiple targets for a given microbial agent (i.e., a wide host spectrum) may be highly desirable, even necessary. Biopesticide companies have to have a certain critical mass to be commercially successful; the locally oriented cottage industry model is rarely appropriate. Production scale has to be large enough, and cost efficiencies great enough (in the face of very expensive labor), for sales margins to pay for the effort. Production boils down to the fully loaded cost of a unit of fermentation per hectare of product. Steadily increasing “red ink” and eventual failure has, all too often, been the outcome of an inability to meet these challenges. These concepts will be illustrated by the author’s experience with one biopesticide company in the US.

Like the agrochemical industry (albeit on a much smaller scale), the biopesticide industry has undergone consolidation, shuffling of product portfolios, and restructuring of the relationships between basic manufacturers, distribution, and end users. As larger companies have abandoned the “life sciences” concept (which combined plant health, veterinary, and biomedical businesses), many of the smaller companies devoted to specific biopesticide technologies have struggled to attain profitability, most without success. At the same time, developments in microbial control technology too often become solutions looking for markets, especially in developed economies. We have had to adjust our thinking on such things as the concept of “big” versus “small” companies, the relative value placed on different characteristics of biopesticides, and how they compare to new synthetic pesticides. Some of these developments could lead to increased adoption of microbial products if they also lead to greater understanding of the economic and technical reasons why certain products or companies succeed while others fail. Recent discussions of the impediments to more rapid adoption of microbial and other biopesticides have been frequent and not without controversy. This presentation is not specifically intended as another discussion of those barriers, but will instead focus on the recent evolution of the biopesticide industry, realities of the marketplace, and what might be learned by comparison with other industries.

**SYMPOSIUM (Div. of Viruses). Monday, 8:00-10:00.**

**Insect resistance mechanisms to viruses: Beyond the midgut**

**Symposium. Monday, 8:00**

**Clues from viral genomes to insect anti-viral immune responses**

Bruce A. Webb

Dept. of Entomology, Univ. of Kentucky, Lexington, KY 40546, USA

Insect anti-viral immunity is so poorly understood and studied as to have suggestions in the literature that it does not exist. However, insects are clearly differentially susceptible to viruses and some insects do mount physiological responses to virus infection. This can only occur if there are mechanisms that convey resistance to virus infection. This presentation will consider the evidence for insect anti-viral immune responses from selected systems described in the recent literature. The literature summary will focus on the potential roles of the melanization, apoptotic and non-productive infection of hemocytes in anti-viral immunity. In addition, I will describe the evidence for anti-viral immune responses as a contributing factor to silencing polydnavirus gene expression in non-permissive hosts. This overview will then consider the evidence for anti-viral immunity that are implicit in the differential responses of insects to polydnavirus infection. Insects and cells that are permissive to polydnavirus infection may show little or no gross pathology. Other cells in the same organism may exhibit gross pathologies including widespread apoptotic response to infection. In insects that are non-permissive, silencing of polydnavirus gene expression is correlated with recovery of the melanization response. Finally, I will consider the immune systems that appear likely to be affected by polydnavirus genes based on patterns of gene expression and identification of viral gene families by genome sequence analyses.

**Symposium. Monday, 8:25**

**Luteovirus transmission barriers in aphids**

Stewart Gray1, Frederick Gildow2, Diana Cox-Foster3, Marina Caillaud4


The luteoviruses do not replicate in their aphid vectors, but the transmission process requires that the virus circulate through the gut and salivary tissues as well as survive in the hemolymph. The circulation pathway is common to all luteoviruses, but the success of completing the cycle can be very aphid species–virus isolate specific. The gut can pose an entrance barrier, although no gut escape barrier has been identified. Virus can easily pass through the gut associated basal lamina into the hemocel. The long-term survival in the hemolymph involves a unique association of the virus with a bacterial endosymbiont protein that seemingly allows the virus to escape attack by the aphid immune system. The salivary gland possesses two potential entrance barriers, the basal plasmalemma is an obvious one, but the basal lamina is even more restrictive. Some viruses are unable to bind the basal lamina, while others are recognized and bind to putative surface receptors, but are unable to move through the matrix. The size exclusion limit of the salivary gland basal lamina is less than the virus diameter suggesting that transport in competent vector species is active rather than passive. Genomic and proteomic technologies are defining the virus components involved in the transmission process, but the aphid remains more of a black box. Biochemical approaches have defined virus-binding proteins specific to vector species and traditional genetic studies have indicated that transmission competency is genetically controlled and that different sets of alleles are involved for different luteoviruses. A growing interest in insect genomics, including aphid genomics, should allow theories of genetic components that define vector competency in aphids and allow the development of novel disease control strategies aimed at reducing virus transmission and disease incidence spread.

**Symposium. Monday, 8:55**

**Aptosis as a defense response against virus infection in insects**

Thomas E. Clarke, Louis Heaton, and Rollie J. Clem

Molecular, Cellular, and Developmental Biology Program, Division of Biology, Kansas State University, Manhattan, KS 66506 USA

Aptosis is a common response of cells to various stress stimuli. Many insect cells appear to be constantly poised to undergo apoptosis, which has been suggested to be an ancient defense response against virus infection. We are examining the ability of apoptosis to thwart baculovirus infection in lepidopteran insects, taking advantage of a mutant of Autographa californica M nucleopolyhedrovirus (AcMNPV) that lacks the anti-apoptotic gene p35. In cells from the fall armyworm Spodoptera frugiperda, infection with p35 mutant AcMNPV results in apoptosis, while infection with wild type AcMNPV does not, due to the ability of the p35 protein to inhibit caspases. However, for unknown reasons cells from the cabbage looper, Trichoplusia ni, are highly resistant to numerous apoptotic sti-
muli, and infection of T. ni cells with either wild type or p35 mutant AcMNPV does not result in apoptosis. These host-virus combinations provide an excellent model system to study the effects of apoptosis on virus infection. S. frugiperda larvae are extraordinarily resistant to infection with p35 mutant AcMNPV by intrahemocoelic injection, requiring approximately 1000-fold higher doses of the mutant virus to result in 50% lethality than wild type virus. In contrast, T. ni larvae are equally susceptible to wild type or p35 mutant AcMNPV. Infection of S. frugiperda larvae with p35 mutant AcMNPV also results in apoptosis in vivo as determined by TUNEL staining and the widespread presence of pycnotic nuclei in infected tissues. These results support the hypothesis that apoptosis can be an effective defense against baculovirus infection. We have recently begun to analyze the response of midgut epithelial cells to infection with p35 mutant AcMNPV, and results from these studies will be presented.

Symposium. Monday, 9:15

Virucidal activity against HzSNPV in 

Holly J.R. Popham, Kent S. Shelby and Sandra L. Brandt

USDA ARS Biological Control of Insects

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Lepidopteran larvae are known to resist baculovirus infection by selective apoptosis or sloughing off of infected cells from the midgut. Once the baculovirus infection breaches the midgut barrier, however, there are few known mechanisms to account for the resistance and clearance of infection observed in some virus/host combinations. For example, encapsulation and melanization of AcMNPV infective foci in tracheoblast cells of H. virescens and M. sexta have been reported, and these processes were inhibited by prior polynucleoside infection or parasitization. Phospholipase of H. virescens has also been reported to inactivate several viruses in vitro. We tested the hypothesis that a factor(s) present in the plasma of infected pest larvae could act to limit the spread of baculoviruses within the hemocoel. We have developed an in vitro bioassay in which Heliocoverpa zea single nucleopolyhedrovirus (HzSNPV) particles are incubated with plasma collected from uninfected Heliothis virescens larvae. The TCID50/ml (50% tissue-culture infectious dose) of surviving HzSNPV were then titered on HzAM1 cells. In vitro incubation with diluted plasma from larval H. virescens exhibited a virucidal effect against HzSNPV, reducing the TCID50/ml by more than 40 fold (7.7 ± 3.6 x 10^3 to 1.8 ± 1.3 x 10^3). The virucidal activity was freeze-stable but heat- and protease-labile. Activity was highest in plasma from early fourth instar larvae. We will report on the biological and biochemical characterization of this constitutive humoral antiviral resistance mechanism in insects, and the linkage of this activity to the inducible antimicrobial response.

Symposium. Monday, 9:40

Intra-stadial developmental resistance of 
gypsy moth to its own baculovirus

Diana Cox-Foster, Mike Grove, Shengzhong Su, James McNeil and Kelli Hoover

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Fourth-instar gypsy moth (Lymantria dispar) becomes markedly more resistant to its host-specific baculovirus, L. dispar nucleopolyhedrovirus (LdNPV) as the insect ages within a stadium. This resistance cannot be overcome by bypassing the midgut and delivering the virus directly into the hemocoel. We report here that larvae were able to clear virus, and this was markedly more pronounced in insects inoculated at the most resistant stage. Larvae were inoculated intrahemocoecically with budded virus of a recombinant of LdNPV that expresses lacZ under control of the hsp70 from Drosophila (LdNPV-hsp70/lacZ) either immediately after molting (4’s) or at 48 hours post-molt. Insects were bled at 24-hour intervals until larvae began to die (Day 11). Hemolymph from each insect was measured for (1) expression of lacZ in hemocytes and (2) progeny BV titer in cell-free plasma by plaque assay. In both 4’s and 4’s, evidence of viral infection (hemocytes signaling lacZ and infectious BV) was detected at 3 days post-infection (dpi), but in both stages the proportion of insects having infected hemocytes decreased late in infection. Also, virus titers dropped to undetectable levels during the course of infection in 4’s and 4’s, whereas infection progressed in 4’s stage larvae. Our data suggest that both stages of insects can overcome viral infection by some form of immune response and/or apoptosis, but that resistant-stage insects clear virus far more effectively. We hypothesize that clearing of virus occurs by induction of immune responses against the virus itself or against virally-infected tissues. A potential immune response is indicated by 1) hemocytes encapsulating the tracheal system servicing the midgut, 2) chemical immunosuppression by diethyldithio-carbamic acid decreasing developmental resistance in a dose-dependent manner, and 3) an increased activation of enzymes associated with immune responses. Intrastadial developmental resistance also involves host tissues becoming refractory to infection and/or failure of hemocytes (and/or other tissues such as fat body) to amplify the virus. 13% of 4’s and 33% of 4’s stage larvae contained hemocytes signaling lacZ without a detectable BV titer. This suggests that in these larvae hemocytes take up the virus, it is transported to the nucleus and uncoats, but there is a block in viral replication (or budding from the cell) that prevents production of infectious BV. Preliminary studies indicate that these responses are hormonally mediated. Published data suggest this phenomenon occurs in other insects.

BACTERIA – 2

Contributed papers. Monday, 8:00-10:00.

Enduring toxicity of transgenic Anabaena expressing mosquito larvicial genes from Bacillus thuringiensis subsph. israelensis Robert Manasherob,1,2 Zacharia Ngalo Otieno-Ayayo,1,4 Eitan Ben-Dov,1,2,4 Rina Miuskovskaya,1,2

Sammy Bousiba1 and Arieh Zaritsky1,3

1Dept. of Life Sciences and 2Microalgal Biotechnol. Lab., Ben-Gurion Univ. of the Negev, POB 653, Be'er-Sheva 84105, Israel; 3BioSan Ltd., POB 3, Arad 44837, Israel; 4Dept. of Mathematics, Envir. & Natural Sci., Solusi Univ., PO Solusi, Bulawayo, Zimbabwe

Persistence of biological control agents against mosquito larvae was tested under simulated field conditions. Mosquito larvicial activity of transgenic Anabaena PCC 7120 expressing cry4Aa, cry11Aa and p20 from B. thuringiensis subsph. israelensis (Bti) was compared with Bti itself (as Bacitox products) and found better when either mixed with silt or exposed to sunlight outdoors. Reduction of Bactimos toxicity against 3rd instar Aedes aegypti larvae was at least 10-fold higher than Anabaena's following mixing with silt. Enduring of toxicity (over 50% mortality) in outdoors experiments (affected by sunlight intensity and temperature), 2-4 days for Bactimos, was 4-16 days when delivered by Anabaena. The difference in residual activity was extended to 10-fold (3 and 30 days, respectively) when 30% mortality was considered.

Contributed paper. Monday, 8:15

Diamondback moth vs. Bt-B. rapa/Br-B. rapa: Who will win? L. Braun1, S.I. Warwick2, P. Mason2, B. Zhu2 and C. N. Stewart Jr.1

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We have completed two years of field and laboratory experiments to determine the effects of herbivory on survival of plants expressing an insecticidal gene. Nine lines of GFP-Bacillus thuringiensis cry1Ac canola (Brassica napus) were hybridized with three wild accessions of bird’s rape (B. rapa) populations. F. hybrids and BC, progeny showing the presence of the GFP-Bt transgenes were backcrossed

– 39 –
with the appropriate B. rapa parent to produce BC1 and BC2 respectively.

Laboratory colonies of local diamondback moth (DBM) populations in eastern and western Canada were established, and bioassays performed to determine the LD90 and LD50 values of Safer's BTK\TM Biological Insecticide (Bacillus thuringiensis subsp. kurstaki) against 2nd and 4th instar DBM larvae. Mean mortality at 10-14 days post-treatment was 100% for DBM neonate larvae fed leaf disks of Bt-transformed B. napus or B. rapa plants (including B. rapa (B. napus F1 hybrids, BC1, and BC2 plants). However, mean mortality at 5-7 days post-treatment for 4th instar larvae fed Bt-transformed B. napus and B. rapa was 73% and 80% respectively. Survival of late instar DBM larvae reinforces the importance of co-deployment of a well-defined resistance management program with delivery of the transgenic plant strategy.

Caged field trials determined effects of GFP and/or Bt genes on plant fitness in B. napus and B. rapa (BC1 lines) under herbivore pressure from DBM. Feeding damage was assessed twice during the season, plants were harvested before seed maturation, and their vegetative and reproductive components weighed separately. Under high insect pressure from DBM, mean reproductive plant weight of lines containing GFP-Bt was significantly higher than that of non-transformed B. napus cv. Westar or B. rapa. Mean reproductive plant weight of canola plants transformed with GFP alone was not significantly different than that of Westar. The expression of Bt conferred a fitness advantage to both the crop (B. napus) and a weedy wild relative (B. rapa).

Contributed paper. Monday, 8:30

Emerald ash borer susceptibility to Bacillus thuringiensis var. kurstaki EG7673
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The emerald ash borer (EAB), Agrilus planipennis Fairmaire (Coleoptera: Buprestidae), a native of China, Japan, Korea, Mongolia and eastern Russia, was discovered killing ash trees (Fraxinus spp.) in >2000 mi\textsuperscript{2} in Michigan and Ontario in 2002; a small infestation was discovered recently in northern Ohio. EAB likely arrived in North America from Asia in wood packing materials ca. six to ten years ago and gradually spread unnoticed due to a general decline of ash in the area. Federal and state agencies are planning to attempt eradication of this invasive pest because urban and forest ash throughout North America are threatened. The extent of the problem, abundance of ash, and lack of knowledge about EAB, suggest eradication will be very difficult. To expedite the development of control methods for EAB, we are bioassaying EAB adults with registered Bt bioinsecticides. Novodor\textsuperscript{\textregistered}, formulated with Bt var. tenebrionis and its Cry3Aa1 toxin, had no effect on EAB adults. However, Raven\textsuperscript{\textregistered}, formulated with Bt var. kurstaki EG7673 which produces Cry3Aa, Cry3Bb, and Cry1Aa, caused immediate feeding inhibition and mortality of EAB adults within 3-5 days. Further research on Cry toxicity in EAB and the potential use of this product for EAB management will be discussed.

Contributed paper. Monday, 8:45

Diversity of bacteria associated with the gut of stem boring beetles (Coleoptera: Cerambycidae, Scolytidae)
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The gut bacterial community of the asian longhorned beetle, Anoplophora glabripennis (Motschulsky), linden borer, Saperda vestita Say, southern pine beetle, Dendroctonus frontalis Zimmermann, and pine engraver, Ips pini (Say), were characterized by molecular methods. Pooled gut samples from larvae of each species were used for DNA extraction, except for pine engraver, from which guts of adult insects were used. 16S rRNA genes were directly amplified from DNA extracted from the guts and cloned, and the clones were compared by examining the patterns generated after digesting them with restriction enzymes. The highest diversity of distinct restriction digestion patterns, operational taxonomic units (OUT), was observed in the gut of asian longhorned beetle and the lowest in one southern pine beetle sample. Patterns of clones assigned to different OTUs was different within and between insect species. Ninety-two clones having distinct patterns were sequenced to determine the similarity of the 16S rRNA genes of the gut bacteria to known sequences from DNA databases. All 16S sequences from southern pine beetle and linden borer and all but two sequences from pine engraver gut bacteria belong to the -Proteobacteria division. The asian longhorned beetle gut community is composed of members of diverse groups such as low G+C gram-positive bacteria, firmicutes and actinobacterium. Several 16S rRNA genes amplified from the gut of all insect species consisted of sequences not previously described. Using aerobic cultivation and medium containing carboxymethylcellulose or filter paper as the sole sources of carbon, we isolated strains of cellulolytic bacteria from the gut of linden borer larvae. Analysis of 16S rDNA sequences showed that these strains have greater than 99% similarity with Sphingomonas yanoikeyae.

Contributed paper. Monday, 9:00

Preliminary observations on effects of Bt-corn on non-target soil Collembola
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Integration of biologically-based pest management techniques into crop protection programs is critical to the development of more sustainable agricultural production systems. Such control agents include biopesticides based on bacteria and fungi, and genetically-modified crop plants expressing insecticidal toxins. To ensure that they have minimal ecological impact, their effects on non-target beneficial soil fauna need to be determined. Collembola play a vital role in the removal, breakdown and re-cycling of crop residues. Stable and abundant communities of these microarthropods are generally present in well-managed agricultural soils and they are now recognized as key indicator species of soil fertility and health. Through laboratory and field tests on these organisms, we can begin to quantify the level of risk posed by biopesticides and transgenic plants. Collembola are frequently abundant in the root zone of plants; with transgenic crops such as Bt corn, they would, potentially, be exposed to toxins secreted into the surrounding rhizosphere by actively metabolizing roots. Furthermore, as Collembola are principally involved in the decomposition of organic matter, they would be exposed to relatively high levels of Bt toxin remaining in crop residues, which are incorporated into the soil. In an effort to document any side-effects such crop protection technologies might have on these non-target organisms, field trials were initiated in 2002, in which plots were planted with Bt-transgenic and isogenic (parent line) silage corn. Soil core samples were taken at monthly intervals (June through October) in the root zone of the corn plants, from the seedling stage until after harvest, and subject to extraction using Berlese funnels. Information on Collembola species diversity and abundance over time will be presented. Root samples have also been taken every month (June 2002 thru April 2003), preserved by freeze drying, and powdered. Root powders have been presented to F. candida in laboratory feeding assays, and effects on fecundity (egg production) and longevity monitored over 6 weeks. Results of these trials will also be presented. Such trials allow some ecological impacts of GMOs to be assessed on a scientific basis, and the relative risks posed by these technologies compared to those posed by existing crop protection strategies.
Contributed paper. Monday, 9:15

Comparative analysis of efficacy of different strains of Bacillus thuringiensis subsp. thuringiensis against Tortrix viridana (Lepidoptera, Tortricidae) in field conditions

Anatoly V. Ivashov, Andrei P. Simchuk, Irina G. Peletskaya and Svetlana Y. Gouli

Application of the microbial formulations for pest control has significant importance for safety of the human health, and conservation of natural environment. It is very important for Crimean peninsula (Ukraine) with unique natural and climatic conditions. Our study was performed to investigate efficacy of different strains of Bt subsp. thuringiensis against oak leaf-roller moth—Tortrix viridana. It is the most important pest in Crimean oak-grove as a rule located on the mountain slopes having tendency to erosion. The study was done in the natural population of T. viridana on the southern coast of Crimea peninsula near Yalta city. Nine model trees of the pubescent oak (Quercus pubescens Willd.) were chosen as models. The canopy of each tree was divided into two parts. One part was spread by microbial formulation, and second served as control. Three different strains of Bt were used in the experiment (BG, B3 and B10). Densities of the T. viridana larvae on each of the model trees were measured before application of microbial formulation and then on seventh day after the treatment. Analysis of variants has shown statistically significant changes of the insect density in experimental variants (F = 28.8; P = 0.00017), while in corresponding controls changes in density were non-significant. Also, the larvae density in experimental variants significantly differed from control variants in seven days after treatment (F = 32.36; P = 0.0001). In spite of this, different strains of Bt have shown different efficiency (F = 4.54; P = 0.034). Correlational analysis of the data obtained show density dependence of density changes both in experimental and control variants (Experiment: R = -0.967; P < 0.01; Control: R = -0.843; P < 0.01). At the same time, density changes in experimental variants positively correlated with changes in control variants (R = 0.806; P < 0.01). These results demonstrate that the processes (leaded to larval mortality) occurred both in experiment and control were the same directed but much more expressed in the experimental variants. As mortality is a consequence of “struggle for existence”, we may conclude that microbial formulations increase in natural selective pressure occurred in the insect population.

Genomic response of C. elegans to Bt crystal protein intoxication

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Bacillus thuringiensis is a gram-positive bacterium that produces pore-forming crystal proteins lethal to invertebrates. These proteins are used around the world (e.g., by the WHO) to control disease-carrying mosquitoes and are expressed in transgenic crops to control caterpillar pests. Using the nematode C. elegans, we are studying in depth the mechanism of pathogenesis of crystal toxin proteins. To gain a global picture of how hosts respond to pore-forming toxins, we are using C. elegans Affymetrix gene chips to uncover the details of how C. elegans responds to Cry5B at the transcriptional level after zero, one, two, four and eight hours of exposure to toxin. Overall, we have identified more than 1,000 genes that are >2X over or under expressed in the presence of E. coli produced Cry5B toxin. Some of these genes may be regulated by the C. elegans host in an effort to defend against toxin activity. Other genes may be regulated in response to the toxin and aid in the intoxication process that leads to death. One up-regulated gene encodes the C. elegans homolog of a signal transduction gene implicated in innate immune responses in mammals following infection by pathogenic bacteria. Indeed, we have functionally demonstrated that this gene is also required for the nematode to mount a significant defense against the pore-forming toxin. Our findings potentially broaden our view of the role of this pathway to include not only defense against a bacterium but also against a bacterial toxin. We are now using RNA interference to further uncover which of the other Cry5B responsive genes also play a functional role in pathogenesis by scoring RNAi mutants for changes in susceptibility to intoxication.

Molecular diagnostics and phylogenetic analysis of Quahog Parasite Unknown (QPX)

Nancy A. Stokes1, Lisa M. Ragone Calvo1, Kimberly S. Reece2 and Eugene M. Burreson3

Contributed paper. Monday, 9:30

Molecular diagnostics and phylogenetic analysis of Quahog Parasite Unknown (QPX)

Kimberly S. Reece2 and Eugene M. Burreson1

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Quahog Parasite Unknown (QPX) is a protistan parasite that causes disease and mortality in the hard clam, Mercenaria mercenaria. QPX has been reported in cultured hard clam populations in New Brunswick, Nova Scotia, and Prince Edward Island, Canada and in Massachusetts, New Jersey, and Virginia, USA. The parasite is primarily found in clams older than about 1.5 years and has caused severe clam mortalities (>80%) in some areas. DNA-based molecular diagnostics, DNA probes and polymerase chain reaction (PCR) primers, were developed for two levels of detection specificity: for members of the phylum Labyrinthomycota and for QPX specifically. These tools targeted different regions of the small sub-units ribosomal RNA (SSU rRNA) gene. The general labyrinthomycte primers amplified DNA from QPX and the thraustochytrids Schizochytrium aggregatum, Thraustochytrium aureum, and T. striatum, but not from M. mercenaria. The QPX PCR primers detected as little as 20 fg QPX genomic and amplified DNA from QPX, but not from S. aggregatum, T. aureum, T. striatum, or M. mercenaria.
Field validation of the QPX-specific PCR assay was conducted over a 16 month period, using 224 clams collected from a QPX endemic site in Virginia. Detection of the parasite by PCR assay was equivalent to histological examination, the established diagnostic method for this parasite. Oligonucleotide DNA probes were evaluated for in situ hybridization assays of cell smears and paraffin-embedded tissues. The labyrinthulomycete probe hybridized with QPX and the three thraustochytrids, with no background hybridization to clam tissue. Two DNA probes for QPX were specific for the parasite but offered limited sensitivity when used independently; however, when used together as a probe cocktail, sensitivity was greatly enhanced. The probe cocktail hybridized with putative QPX organisms from Virginia, New Jersey, Massachusetts, and New Brunswick. SSU rRNA gene sequences were obtained for these geographically distinct QPX organisms. Phylogenetic analyses based on the QPX and Labyrinthulomycota sequences confirmed earlier reports that QPX is a member of this phylum, but could not definitively demonstrate that all of the QPX organisms were the same species.

Symposium. Monday, 11:10
The first occurrence of MSX disease in Canada – aberrant pathology and discovery of SSO
Sharon E. McGladdery, Mary F. Stephenson, Nellie Gagné and Andrea Locke
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The first occurrence of MSX (Haplosporidium nelsoni) disease in Eastern oysters (Crassostrea virginica) was detected in Atlantic Canada in October 2002. It was associated with mortalities of ~80% in market-sized adult oysters, 2-4 years old, from St. Patricks’s Channel, Bras d’Or Lakes, Nova Scotia. This is a unique hydrographic water body with 20-28 ppt salinities in summer fed by very limited deep seawater exchange with the entrance to the southern Gulf of St. Lawrence. Histology revealed plasmodia and spores in adult oysters at sites with the most severe mortalities. The identity of the parasite was confirmed by the Office International des Epizooties (OIE) reference laboratory for Haplosporidiosis and Perkinsiosis at the Virginia Institute of Marine Science (VIMS). An intensive survey was initiated in collaboration with the provinces, industry and First Nations stakeholders between October to December 2002, and affected areas were placed under stringent harvest controls. Results indicate that the heaviest infections appear confined to oysters within Bras d’Or Lakes, however, infections were also found in oysters showing no clear evidence of mortalities at neighbouring sites. These were linked by oyster transfers for seed collection or depuration relay. Subsequent sampling of surrounding wild populations, also revealed proliferating plasmodial infections within St. Patrick’s Channel. Additional samples collected from the northern coast of Cape Breton and from various locations within the southern Gulf of St. Lawrence revealed light plasmodial infections, with no obvious associated pathology. Although these plasmodia resembled MSX, pathology, intensity and prevalences of infection were significantly different from those detected within Bras d’Or Lakes. Subsequent analyses of these ‘light’ infections using both MSX and SSO PCR-probes revealed that these infections were due solely to SSO, making this another northern extension of the previously reported geographic range of this oyster parasite. This identification was also confirmed by VIMS. Since October over 3000 oysters from 30 sites, along with 150 mussels from the apparent focus of MSX infection have been examined histologically. Suspect negative samples and samples with plasmodial stages that could not be readily identified to species were further examined using PCR based gene probes. The results from these analyses, and apparent over-winter infection dynamics in Canadian waters will be presented and discussed in light of established infection dynamics in eastern US waters. In addition, implications for both national and international mollusca disease controls will be presented, including and potential point source being linked to other water users.

Symposium. Monday, 11:30
Development of biochemical indicators of stress for bivalves: Recent studies on heat shock proteins and proteases
Neil Ross1, Emmanuel Egbossima1, Nicole Brun1,2, Monica Bricelj1, Thomas MacRae1, Joanne Harding1, Cyr Couturier1, and Jay Parsons1
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The goals of our research are to examine and correlate potential biochemical indicators of stress in order to further understand the stress response at the biochemical level and to provide tools to the shellfish industry and research community for assessing culture and harvesting practices. By understanding the bivalve stress response at the biochemical level, we may be able to mitigate a stressful situation and provide the animals with a chance to recover (and avoid death), and, as well, to potentially allow the animals to adapt to subsequent stressors, including those that may otherwise have be lethal (e.g., acquisition of thermotolerance). The first example of the work we are carrying out is the correlation of the neutral red retention (NRR) time and the level of a metalloprotease in the hemolymph of Mytilus edulis acclimated at 5°C and subjected to 10°C temperature shock. NRR assay of hemocytes showed a progressive decrease in retention time up to 9 h, and a recovery to pre-stress values at about 24 h. This change negatively correlated (R=−0.88) with the level of a 55 kDa hemolymph metalloprotease. We propose that this metalloprotease was released from hemocyte into the cell-free hemolymph following temperature shock and that levels of the cell-free hemolymph metalloprotease may be an indicator of temperature shock induced stress in mussels. In a second project, we are examining HSP 70 expression in mantle tissue of juvenile sea scallops (Placopecten magellanicus) and juvenile bay scallops (Argopecten irradians) subjected to acute heat shock (10°C increase for 3h), and in bay scallops and hard clams (Mercenaria mercenaria) following acute cold shock (17°C decrease for 3h). Interestingly, we observed no differences in HSP 70 expression in heat-shocked sea scallops over 24 h. In contrast, HSP 70 levels in bay scallops increased significantly during and following heat shock, attaining a maximum by 12 h, and exceeded control levels even after 8 days. The difference in HSP response may be indicative of the adaptability of these scallop species to environmental perturbations. In bay scallops and hard clams subjected to acute cold shock, HSP 70 levels increased significantly in both bivalves, with levels still increasing after 8 d and 24 h respectively. The duration of the stress response to acute temperature shock may have application in acquired thermotolerance of bivalves transferred from hatchery to field growout sites or to protection from diseases. In future, we plan to examine the linkage of the bivalve stress response with responses to disease.

Symposium. Monday, 11:50
Fixed phagocytes of the digestive gland - A mostly ignored part of the immune system of lobsters (and other crustaceans)
Jan Robert Factor
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Fixed phagocytes of decapod crustaceans are tissue-bound phagocytic cells that together constitute an important defensive organ. First recognized in the early twentieth century by Cuénot as "l'organe phagocytaire", his work appears to have been forgotten for many years. Circulating phagocytic hemocytes have received the primary attention as the mechanism of cell-mediated immunity, yet the fixed phagocytes must be included in our consideration of crustacean defenses. Typically, there is a large population of fixed phagocytes in the digestive gland (hepatopancreas) of the American lobster (Homarus americanus) and other decapod crustaceans. The phagocytic cells are organized as nodules, rosettes, or a layer surrounding terminal branches of the hepatic artery. These terminal arterioles lie in the hemal sinuses among the digestive tubules, and are bathed in circulating hemolymph as it percolates through the digestive gland.
A new disease of lobsters that caused mortalities in wild lobsters during summer 2002 is described as a form of calcnosis. A significant number of moribund and dead lobsters were reported to state authorities by lobster fishers in Long Island Sound, New York, during the summer of 2002. Moribid lobsters were characterized by an orange discolouration of the abdomen, lethargy, an excess of epibions and poor post-capture survival. Affected lobsters displayed a significant coagulopathy marked by a lack of clotting. Severe extensive multifocal or diffuse mineralized granulomatous inflammation of the gills and intestinal walls was the most striking pathology. In the gills, granulomas were frequently seen to be lodged in filaments, resulting in congestion, ischemia and coagulative necrosis of gill tissues. In the antennal glands, granulomas were concentrated along the border between the filtration and resorption zones of the organ. Affected lobsters lacked observable reserve inclusion cells (energy storage cells) and thus appeared to be either malnourished or metabolically exhausted. No significant pathogens were recovered from diseased individuals, suggesting that the disease is of metabolic origin. In lobsters with early stage disease, it was evident that granulomas were focused around calcium carbonate crystals consistent with the mineral form aragonite. Aragonite crystals were identified by their spheroid shape, radial striations, clear to golden brown colouration and strong birefringence. In early stage individuals, naked aragonite crystals were observed, whereas in later stage individuals, aragonite crystals were observed to be at the centre of granulomas. In most cases, the granulomas had continued to mineralise in an amorphous fashion. It is not yet clear why this disease occurs but it may be related to anomalously high sea bottom temperatures in Long Island Sound (~23°C) during the summer of 2002 and associated disruptions of the calcium and respiratory chemistry of lobsters in favour of deposition of calcium minerals in soft tissues from the blood will be explored.

Contributed papers. Monday, 10:45
Evaluation of entomopathogenic nematode strains for the control of Anoplophora glabripennis
D. Fallon1, L. Soltér2, M. Keena2, J. Cate3, M. McManus3, and L. Hanks4

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Six strains of entomopathogenic nematodes (EPNs) were screened for efficacy against the Asian longhorned beetle (ALB), Anoplophora glabripennis. Four steinernematids; Steinernema feltiae SN, S. carpocapsae TX, S. riobrave SN, and H. marelatus IN were used in two bioassays to screen nematode effects on ALB. A filter paper bioassay using a 24 hour exposure of nematode-to-insect, and a feeding-pot bioassay using a 72 hour exposure of nematode-to-insect were conducted in controlled temperature chambers at 24°C. Each bioassay chamber contained a single ALB larva. Bioassays were conducted using third, sixth, and seventh instar ALB larvae. EPNs were applied at 100 JJs / larva. Each treatment had 7 replicates. An additional bioassay was conducted using 10 neonates and 100 JJs in wells of a 24-well culture plate. Neonate larvae were susceptible to all isolates screened using a filter paper bioassay, mortality ranged from 97% by S. feltiae SN to 39% by H. marelatus. Third instar larvae were susceptible to all isolates screened in the filter paper bioassay; S. feltiae SN and S. carpocapsae Sal were the most effective causing 100% mortality. In the feeding-pot bioassay, only S. feltiae SN and S. carpocapsae were effective, killing 100% of the larvae, sixth and seventh instars were similarly susceptible to S. feltiae SN and S. carpocapsae Sal, but the remaining isolates screened were ineffective. Nematode preconditioning to aqueous ALB frass did not enhance larval mortality. However, S. feltiae SN juveniles were positively attracted to ALB frass-extracts favoring its use in locating ALB larvae in cryptic environments like bore chambers or bark. Our results demonstrate the potential use of S. feltiae SN and S. carpocapsae as control agents for ALB.

Contributed paper. Monday, 11:00
Susceptibility of the European crane fly to four entomopathogenic nematodes (Steinernematidae and Heterorhabditidae)
Louis Simard1, Guy Belair2 and Julie Dionne3

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Larvae of the European crane fly (Tipula paludosa), commonly called leatherjacket were reported for the first time on Quebec’s golf courses in 2001. This new emerging insect pest was observed on tees, fairways, roughs and greens. Turf damage is more important on greens and tees consequently the threshold is low. Moreover, European crane fly is known to cause recurrent damage on lawns and golf courses elsewhere in Canada including Ontario, the Maritimes, and British Columbia. Insecticides registered in Canada for leatherjacket provide good control of this pest. Recently, the Quebec Government banned pesticide uses on residential lawns and park and constrained golf courses to reduce their pesticide applications. In this situation,
entomopathogenic nematodes represent a good alternative to pesticide and they have potential to take a more important place in the Canadian turf industry. Our objective was to assess the virulence of four entomopathogenic nematode species against the leatherjacket in Quebec. In the laboratory, full-grown larvae were exposed to different concentrations (0, 200, 700, 1200 and 7000 nematodes per insect larva) of Heterorhabditis megidis, H. marelatus, Steinernema carpocapsae, and S. feltiae. These treatments were applied to both actively feeding larvae on turf and larvae with no turf. Experiments were performed in transparent plastic containers at 24°C for a 5-day exposure of larvae to nematodes. Mortality counts were done at 5 and 10 days. For all nematode species, higher mortalities of leatherjacket were obtained when larvae were actively feeding. At 200 nematodes per larva, S. feltiae increased the mortality of the leatherjacket from 5 to 60% in presence of turf. S. feltiae has shown a significantly lower LC50 value of 153 when compared with 562, 763, 3784, for H. megidis, H. marelatus, and S. carpocapsae, respectively on actively feeding larvae.

Contributed paper. Monday, 11:15

Steinernema scarabaei: ecology and efficacy against white grubs
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Steinernema scarabaei was isolated from epizootics in populations of Popillia japonica (Japanese beetle) and Exomala orientalis (oriental beetle) larvae in turfgrass areas in New Jersey. In laboratory studies S. scarabaei was highly pathogenic to and reproduced well in oriental beetle and Japanese beetle larvae but its pathogenicity to and reproduction in larvae of 4 lepidopteran species was mediocre and variable. Pathogenicity to and reproduction in larvae or adults of species from other families of Coleoptera and other insect orders was low. S. scarabaei is well adapted to infecting sedentary hosts below the soil surface but poorly performs against mobile hosts on the soil surface. S. scarabaei caused significant mortality to and reproduced in oriental beetle larvae at 15 to 27.5°C.

In the laboratory, S. scarabaei was highly pathogenic to 3rd instars of P. japonica, E. orientalis, Rhizotrogus majalis (European chafer), Maladera castanea (Asiatic garden beetle), and 3 Phyllophaga spp. (May/June beetles). In contrast, S. glaseri and Heterorhabditis bacteriophora were very pathogenic to P. japonica larvae but showed mediocre to very low pathogenicity to the other above species. All 3 nematodes showed only mediocre pathogenicity to 3 Cyclocephala spp. (masked chafers) and low pathogenicity to the Cotinis nitida (green June beetle). However, in microplot field trials (2.5x10⁹ nematodes/ha; 21 DAT), S. scarabaei provided 71-100% control of P. japonica, E. orientalis, R. majalis, M. castanea, and C. borealis (northern masked chafer). H. bacteriophora provided 90% control only against P. japonica but 10-50% control of the other white grub species.

To test long-term effects, we treated 1.5 m² turfgrass enclosure containing 160 E. orientalis larvae at rates of 0, 0.4, 1.0, or 2.5x10⁹ S. scarabaei per ha. At 31 DAT, every larva recovered in the 3 densities were S. scarabaei-infected. Based on previous studies, this high efficacy could only have been achieved through additional infections caused by nematodes emerged from hosts infected by the originally applied nematodes. This was supported by increased S. scarabaei-densities as determined by saturation baiting of soil samples.

Contributed paper. Monday, 11:30

The effect of inundative application of entomopathogenic nematodes on soil processes: A microcosm study
Elizabeth A. B. De Nardo,1,2 P. S. Grewal,1 D. McCartney1 and B. R. Stinner1
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Entomopathogenic nematodes (EPNs) and their associated symbiotic bacteria have been considered as a safer approach to pest control than the chemical pesticides. They have been proved to be safe to the humans and several other above and below ground vertebrates and invertebrates. However, some recent studies have indicated that EPNs have the potential to affect the diversity of native fauna in soil ecosystems even though they do not have any direct parasite/host or predator/prey relationship. EPNs are applied often as inundative strategy and repeated applications of these nematodes to control recurring pest populations may sustain the impact. Metabolic products of symbiotic bacteria of EPNs are reported to possess a broad spectrum of biological activities and fundamental questions arise about their impact on soil fauna and flora and consequently affecting soil processes. The impact of an inundative release of Steinernema carpocapsae and the insecticide Trichlorfon (Dylox 80) in the presence or absence of Galleria mellonella larvae, on the soil microbial respiration; microbial biomass (total nitrogen), and mineral nitrogen (NH4-N, NO3-N) were evaluated in a microcosm study. The results from the first trial indicated that treatment with S. carpocapsae with or without G. mellonella larvae do not cause detrimental affect on the soil processes measured. In fact, the EPNs increased the amount of NH4-N, N-NO3, N and microbial biomass (total N) significantly, compared to the pesticide and control treatment, at least until 15 days.

Contributed paper. Monday, 11:45

Differential susceptibility of larval instars of the citrus root weevil, Diaprepes abbreviatus, to the entomopathogenic nematode, Steinernema riobrave
Robin J. Stuart and Clayton W. McCoy
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The root weevil, Diaprepes abbreviatus (L.), originated in the Caribbean and is now a major pest of citrus, other crops and ornamentals in Florida. Young larvae feed on fibrous roots, move to larger roots as they grow, and pupate in the soil after 9-11 instars. We examined the influence of larval age, weight and instar on the susceptibility of D. abbreviatus to the entomopathogenic nematode, Steinernema riobrave Cabanillas, Poinar and Raulston. Diaprepes larvae belonging to different age cohorts were obtained from the USDA rearing facility in Fort Pierce, FL. Each larva was weighed and the head capsule measured according to standard procedures. Larval instar was determined on the basis of head capsule width. Larvae were placed in individual 25-dram snap-cap vials in Candler sand with 8% moisture by weight, and S. riobrave was applied at rates of 100 to 500 infective juveniles per container. Treatment containers were incubated at 24°C and mortality was checked after 9-12 days. Mortality varied significantly among instars and decreased markedly in later instars. Within instars, mortality was not related to larval weight or age. The mechanisms responsible for differential susceptibility of larval instars are unknown but this phenomenon could have implications for the timing of nematode applications for weevil control in Florida citrus.

Contributed paper. Monday, 12:00

Effect of insect food plant and selection on infectivity, sex ratio, and melanization of Steinernema spp. in Diabrotica undecimpunctata howardi
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We conducted assays to determine if the infectivity, sex ratio and melanization of entomopathogenic nematodes in the genus Steinernema varies in response to food plant of the host insect and to selection on a particular insect/host plant combination. Three isolates of Steinernema carpocapsae (Agriotos, Mexican, and a Hybrid) were continuously cultured in corn-fed southern corn rootworm, Diabrotica undecimpunctata howardi, for 25 passages. The selected nema- todes were compared to the same isolates maintained on Galleria mellonella ("unselected"). The infectivity, sex ratio, and melanization of the three rootworm-selected and unselected isolates of S. carpocapsae and unselected S. riobrave were measured in southern corn rootworm that had fed on corn (Zea mays), peanut (Arachis...
results could lead to a more thorough understanding of the relation-

When compared with unselected nematodes, the Agriotes isolate
selected on corn-fed rootworms for 25 passages showed an increase
in infectivity on corn-, non-bitter squash- and peanut-fed rootworms
but not on bitter squash-fed rootworms. The Mexican isolate selected
on corn-fed rootworms showed an increase in infectivity only in corn-
fed rootworms. The corn-selected Hybrid isolate showed an increase
in infectivity on rootworms from all hosts except non-bitter squash.
The proportion of invading nematodes that was melanized by the
rootworm host was generally higher among selected nematodes
compared with unselected nematodes. The proportion of invading
nematodes that was melanized in the rootworm was generally lower
in bitter squash-fed rootworms compared with rootworms that had fed
on other host plants. The proportion of nematodes developing into
males was generally lower among selected nematodes compared to
unselected nematodes. Infectivity of S. riobrave was not affected by
host food plant, but the proportion of invading nematodes that was
melanized was higher in corn- and peanut-fed than in squash-fed
rootworms. The proportion of male nematodes was higher in bitter
squash- and peanut-fed rootworms than in corn- and non-bitter
squash-fed rootworms. These results demonstrate that insect food
plants can affect several aspects of the infection/life cycle of steinernematid nematodes.

**CONTRIBUTED PAPERS. Monday, 10:30–12:30.**

**MICROBIAL CONTROL**

**STU** Contributed paper. Monday, 10:30

**Exploitation of natural enemies and pathogens to activate a persistent baculovirus in field and laboratory populations of the cabbage moth Mamestra brassicae**

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Baculoviruses can cause lethal infections in many Lepidopteran pests and are consequently used as a natural control method worldwide. The detection and prevalence of a persistent, non-lethal baculovirus infection by sensitive molecular techniques has previously been described. This persistent virus is harboured within both laboratory and field populations of the cabbage moth Mamestra brassicae, a serious pest of brassica crops in the UK and throughout Europe. Although normally causing minimal harm to the host, this virus can be activated by infection with another baculovirus resulting in a lethal infection by the persistent virus.

Our research has shown that by threatening the host with a natural enemy or other entomopathogen, the persistent virus can be activated and ultimately kills the host. The solitary endoparasitoid Meteorus gyator infects M. brassicae and other Noctuid pest species. A group of M. brassicae larvae known to harbour a persistent baculovirus were parasitised by M. gyator in a laboratory bioassay. Despite never coming into external contact with this pathogen, 10% of the larvae succumbed to a lethal viral infection whilst the remainder died of parasitic infection. Similarly, infection of the persistently-infected larvae with a low dose of the entomopathogenic fungus Beauveria bassiana can lead to persistent virus activation. These unique findings suggest that virus persistence may play a vital role in the natural control of pest species. Threatening the host activates the persistent virus and we suggest that virus activation may be an escape mechanism for the pathogen ‘trapped’ within its host. Ultimately, this could be exploited as a novel form of pest control and by activating the host’s own enemy from within, rather than from without, pest species such as M. brassicae may be controlled using minimal human input within an IPM programme. In addition, these results could lead to a more thorough understanding of the relationship between entomopathogens and their hosts.

**Contributed paper. Monday, 10:45**

**Improvements in the large scale production of the velvetbean caterpillar, Anticarsia gemmatalis, nucleopolyhedrovirus in the laboratory**

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The NPV of A. gemmatalis (AgMNPV) is currently being used on over 1,600,000 hectares of soybean in Brazil. Although two private companies had previously attempted to produce the AgMNPV in the laboratory, they ceased production because of the high costs involved, specially those related to artificial diet ingredients (mainly agar and casein), rearing recipients and labor. Thus, large-scale field production of the AgMNPV has been the sole method employed presently, but it is highly dependable on host abundance, which is affected each year by abiotic and biotic factors, resulting in variable virus yields each season. The objective of this work was to improve the laboratory mass production and processing of the AgMNPV, so as to turn the final product cost competitive with available chemical insecticides to control the insect. Initial studies were conducted to substitute the agar and reduce the amount of casein previously utilized for A. gemmatalis rearing and AgMNPV production. The use of Carragena GP-911 compared to the “Invitrogen P.A.” and “All Chemistry” agars and reduction of casein by 50% proved successful for virus production, resulting in 86.2 to 95.7% reduction of the diet cost. The modified diet did not alter the survival rate or the weight of caterpillars and pupae. Larvae inoculated as fourth instars with 950,000 OBs/ml of diet in cardboard boxes (30x30 cm and 9 cm high) (350 larvae/box), and maintained at 28°C provided the highest AgMNPV yield. In this conditions, efficiency of virus production was over 75% (with cannibalism being the major cause of yield loss). Laboratory production yielded an average of 58.8 hectares equivalent (HE)/kg of AgMNPV-dead larvae, compared to 40 to 50 HE/kg of dead larvae collected under field conditions. Also, the former method resulted in a much lower level of contaminants. The mechanical extraction of the AgMNPV from dead caterpillars (through an adapted fruit juicer) yielded 92.8% of the virus against 75.6% obtained from the manual extraction. Combining these results, the cost of the final AgMNPV product after its production in the laboratory, processing, formulation, quality control, and packaging was reduced enormously compared to previous procedures, turning the biological insecticide cost competitive with the chemical insecticides. The new laboratory procedures is being proposed to some of the companies producing and commercializing the AgMNPV.

**Contributed paper. Monday, 11:00**

**Trends of mass production of microbial pesticides in Russia**

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In Russia, the first experience for mass production of microbial formulations occurred with the fungus Metarhizium anisopliae isolated by Iliia Mechnikov in 1879. Small-scale production continued for about 25 years but appeared not to be very successful. The first bacterial pesticides, Dendrobacon based on Bacillus thuringiensis (Bt) subsp. dendrolimus (sotto) and Entobacter based on Bt subsp. galleriae, were developed in the 1950-1960s. Production technology for formulation was orientated to industry-scale production and a special factory was built for this purpose in the Novosibirsk region. Viral insecticides were based on nucleopolyhedrosis viruses of gypsy moth (Lymantria dispar L.) and cabbage moth (Mamestra brassicae L.), and were also produced on a large-factory scale. Fungal formulations for pest control were only produced under laboratory conditions attached to local Regional Plant Protection Stations and Greenhouse Associations. From the beginning of the 1990s the situation started to change, and nowadays, various microbial formulations are produced not only in the regional biological laboratories but in small firms (cottage industries) arisen under the new economic conditions. The large Russian factory in Novosibirsk region is now producing formulations based only on different Bt
Can composted mulches create an environment that promotes the incidence and activity of natural enemies for control of avocado thrips in California avocado orchards?

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Avocado production in California has been severely impacted by avocado thrips, Scirtothrips perseae, an invasive species from Latin America. Crop losses resulting from feeding damage by adults and larvae, and additional expenses associated with their control, cost the avocado industry an estimated $8-11 million/ year. This pest has forced growers to move from crop protection strategies that have traditionally been biologically-based to ones that are heavily reliant on insecticides. New cultural and biological control strategies are urgently needed. Approximately 78% of thrips larvae drop from avocado trees to pupe beneath the host plant. Placement of organic mulches under avocado trees can reduce thrips emergence by >50% in comparison to non-mulched plots. We have shown that mulch harbors a diverse community of natural enemies (arthropod predators, entomopathogenic fungi and nematodes). Generally, avocado orchards are bereft of ground cover; mulching appears to create an environment that promotes the incidence and impact of these beneficial organisms. So far, 21 nematode genera have been recovered from mulched plots vs. 11 from non-mulched plots, including one isolate of Steinernema feltiae. Over 600 isolates of entomopathogenic fungi have been recovered; Beauveria bassiana has been the predominant species (>95% of all fungal isolates), followed by Metarhizium anisopliae. Two distinct morphotypes of B. bassiana have been evident, including one that appears to be able to colonize mulch/soil. Selected strains are now being screened for activity against thrips, with a view to their potential development as bioamendments. Data will be presented on the seasonal incidence of thrips and predictions of thrips populations on mulched versus non-mulched plots. Selected strains are now being screened for activity against western flower thrips which is being used as a surrogate host. Use of composted mulch to control avocado thrips would allow growers to return to a more ecologically-sound production system by reducing or eliminating the need for insecticides, while simultaneously promoting orchard health through the biological control of avocado root rot, improved soil fertility, increased water conservation, and weed control.

The definitions and measurement of pathogenicity and virulence

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The terms pathogenicity and virulence are used in many scientific disciplines: including medicine, epidemiology, evolutionary ecology, microbiology, and plant and insect pathology. The definitions of these terms vary both between and within disciplines; the purpose of this paper is to examine the current use of pathogenicity and virulence in the invertebrate pathology literature and suggest changes to promote consistency with other disciplines. We are proposing a hierarchy of terms where Pathogenicity = Infectivity x Virulence. Pathogenicity is defined as the ability to cause disease, which we view as the ability to enter a host, establish within it, and cause disruption in host homeostasis. Infectivity is defined as the ability of the pathogen to enter the host and establish/sustain within the host, and virulence is seen as a measure of the severity of disease.

The entomopathogenic Hyphomycetes fungi have been used for microbial pest control for more than one hundred years. But these pathogens are put into practice in very limited scale. Researchers are attracted by the simplicity of cultivation of Hyphomycetes fungi, mainly a contact mechanism of penetration into the host, a broad spectrum of action and relative ecological safety. However, problematic to its expanded use is its instability in adverse environmental conditions, especially high humidity. For the real enhancement of effectiveness of the fungi for plant protection it is necessary to provide several basic conditions: 1. Conforming optimal cover of the plant and pest body with fungal material. 2. Creating the optimal physical condition during the initial period of action of fungus. 3. Decreasing or eliminating the latent period between application and effect of the fungal formulation. 4. Temporarily creating unfavorable conditions for the natural microbial community. All these conditions can be provided if we use the blastosporas for pest control. The basic
rationale for such an approach is the fact that blastospores are significantly more active than conidia. Without preliminary drying the blastospores form a relatively stability suspension. This suspension covers the plant surface very well without any additional chemical substances. The problem connected with optimal conditions for initial action of the fungal formulation is solved because the blastospores do not have a latent period of action. For the fourth basic condition, it is possible to use as carrier the liquor after cultivation of the fungi. The cultivation liquor contains the complex of biologically active substances providing temporary suppression of the local microbial community in the habitat of the target pest and at the same time promotes penetration of the fungus through protective insect barriers. The fungal formulation based on blastospores will have a much lower price, because the mass-production technology will be significantly more effective owing to complete processing of the nutrient media and the short period of fermentation. The principal problem for realizing this approach is the relatively short period of the viability of blastospores. But our preliminary research shows that under simple conditions these propagules conserve a high level of activity around two months. The half-life period of blastospores is around three months. This time is acceptable for the cottage technologies. Beside that there are numerous possibilities for prolongation of the viability of blastospores.

However the pathogenicity of HpBa-1 against other scrub grubs shows relatively low. We thought that the specificity of HpBa-1 derived from the interaction between immune response of H. picea larva and HpBa-1. In this study, we investigated the immune response of H. picea larva against B. amorpha (Strain: HpBa-1), Metarhizium anisopliae (Strain: PMA-7) and Beauveria brongniartii (Strain: BbB76). Especially, we investigated the adhesion of some fungal conidia on the cuticle and researched changes of the hemocytes number, phenoloxidase activity and protein in hemolymph to some fungi. The adhesion and development of fungi were investigated under fluorescence microscopy and scanning electron microscopy. These microscope studies indicated that HpBa-1 conidia more adhered the cuticle compared with other fungus after treatment of the same concentration. The difference of adhesive ability is one factor of specificity. Furthermore, we researched change of the hemocytes number after each fungal conidia injection. When PMA-7 and BbB76 were injected, the total hemocytes number was maintained control level. Therefore, hemocytes were thought effective immune response to fungi in H. picea larva. But the total hemocytes number decreased after low concentration HpBa-1 injection. When the serum after injected with each fungal conidia was analyzed by SDS-PAGE, specific protein band of approximately 25kDa expressed at 72 hours post injected with HpBa-1. We thought that this protein affected the hemocytes of H. picea larva. From these results, HpBa-1 metabolized the immunosuppressive protein and able to overcome the H. picea hemocytes, but other strains were inhibited by hemocytes effectively. This result was another factor of specificity. These abilities of HpBa-1 were the factor of specific pathogenicity to H. picea larva.

**Beauveria as a possible coffee endophyte**

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The coffee berry borer, *Hypothenemus hampei* (Ferrari) (Coleoptera: Scolytidae), is the most important insect pest of coffee throughout the world. Endemic to Central Africa, it has now spread to most coffee growing regions. Female adults enter the coffee berry and deposit their eggs; larvae feed on the endosperm, lowering the quality of the berry and possibly causing abscission of the fruit. There is a 10:1 female to male sex ratio, and females mate incestuously inside the berry; once the inseminated female emerges from the coffee berry, it is ready to deposit eggs in another berry. This life cycle makes the insect an extremely difficult candidate for control. Insecticides are not a viable option due to their high costs which make them impractical, particularly in view of the historically low coffee prices prevalent today. Recent efforts at coffee berry borer management have relied in biological control alternatives, including the mass release of parasitoids and the use of fungal entomopathogens, but their use is not yet economically feasible. Thus, innovative biological control alternatives are needed. We have initiated a study aimed at establishing *Beauveria bassiana* in coffee. Surveys of endemic coffee endophytes have been conducted in Hawaii, Puerto Rico, Mexico, and Colombia. Dozens of fungi have been isolated into pure culture and are in the process of being identified using nuclear ribosomal ITS sequences. Preliminary results indicate a prevalence of *Colletotrichum* in Mexico and Puerto Rico, as well as the presence of *Parasporobacterium, Lasiidioploea, Articulosporium, Nodulisporum, Xylaria*, and *Phomopsis*. We are also conducting bioassays to assess the virulence of over 50 *Beauveria* strains isolated from the coffee berry borer in various countries. Once the most virulent strain has been identified, it will be the focus of our research aimed at inoculating coffee with *Beauveria*. Various techniques will be used in the inoculation process; microsatellite and other nuclear markers will be used to assess the establishment and movement of *Beauveria* in the coffee plant.

**The specificity analyze of entomopathogenic fungus Beauveria amorpha**

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The pathogenicity of entomopathogenic fungus, *Beauveria amorpha* (Strain: HpBa-1) to *Heptophylla picea* larva indicates especially high.

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**Comparative pathogenicity and genetic variation of *Beauveria bassiana* isolates from Asian longhorned beetle and other cerambycids**

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The Asian longhorned beetle (ALB), *Anoplophora glabripennis* (Motsch.) (Coleoptera: Cerambycidae) is an invasive wood-boring pest attacking hardwood trees in the United States. We are studying the natural enemy complex of ALB in both the US and its native China. *Beauveria bassiana* is the most prevalent entomopathogen of ALB, causing mortality during all life stages. Genetic analyses of 13 *B. bassiana* isolates, collected from infected ALB, cottonwood borer (CWB), *Plectrodera scalator*, and the spotted pine Sawyer (SPS), *Monochamus scutellatus* (Say), were done using polymerase chain reaction-based random amplified polymorphic DNA using 12 primers; differences in colony morphology were also observed. Nine of the 13 *B. bassiana* isolates were distinct with four ALB isolates each from Hebei and Gansu provinces of China and New York City and Chicago in the US; two CWB and two SPS isolates from East Lansing, Michigan. The pathogenicity of one ALB isolate was determined for adults of both CWB and SPS, both native cerambycids, using a standard laboratory bioassay. We found the *B. bassiana* LC50 and LT50 were significantly lower in SPS than in CWB, due in part to the relatively smaller size of SPS than when compared to CWB. Studies on the pathogenicity of this isolate in ALB adults are planned. The suitability of CWB as a surrogate species for the study of ALB management using *B. bassiana* will be discussed.

Poster / Fungi. F-4.

**Efficacy of *Beauveria sp.* in the control of adult Andean Potato Weevil (*Premnotrypes suturalisculus* Kuschel)**

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The Andean Potato Weevil (*Premnotrypes suturalisculus*, APW) is the most serious insect pest affecting potato production in the high Andes, causing up to 50% yield loss through tuber damage. Currently, farmers are using intensively carbamates and organophosphates to prevent damage. Adults infected with *Beauveria sp.* are frequently found in the field. To assess the natural infestation rate of adult, living adults were collected in six different fields (100 adults from each field), placed individually in petri dishes and observed over 2 month for the presence of *Beauveria sp.* Results for the 6 fields were 59%, 8%, 16%, 5%, 6% and 36%. In a field essay, the efficacy of *Beauveria sp.* on adults was tested by directly infecting adults (T1) and potato leaves (T2) by submerging them in a solution of 1 x 10⁸ spores/ml. The weevils were placed with potato leaves below a jute sacking (commonly used as shelter trap for the weevils) within 1 m² surrounded with a plastic barrier to avoid that the weevils escaping. The leaves were changed weekly, exposing the weevils in T2 to the fungus. For the leaf assay, 21% (T2) after 3 weeks and 29% (T1) and 29% (T2) after 6 weeks, compared to 4% and 14% in the control. In a laboratory essay, prelethal effects of *Beauveria sp.* on adult APW were assessed by submerging the adults in a solution of 1 x 10⁸ spores/ml and placing 10 adults (5 females and 5 males) in a receptacle with potato leaves. Mortality was recorded 3 times per week, and eggs were counted and adults weighted over 5 weeks. There were no effects of fungus infection on weight or on oviposition, while mortality by fungus was 10% after 14 days and 42% after 24 days. *Beauveria sp.* may control APW adults in the field though, due to its slow mode of action, it would have to be used in a preventive way.

Poster / Fungi. F-5.

**Comparative virulence of wild type and recombinant vegetatively compatible strains of *Beauveria bassiana* against Colorado potato beetle**

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We developed a system for grouping strains of *Beauveria bassiana* according to the vegetative compatibility of nitrate non-utilizing (*nit*) mutants. We showed that members of one vegetatively compatible group were genetically quite similar. All of these isolates originated in eastern North America and all were associated with Colorado potato beetles. We obtained recombinants of two compatible pairs of isolates (ARSEF 252 x ARSEF 5813 and ARSEF 5813 x ARSEF 6986) by coinoculating beetles and screening fungal progeny from cadavers. We wished to determine whether this form of recombination could alter pathogenicity and virulence. In this study we report the results of a series of dose-response assays to compare parent *nit* mutants and their recombinant progeny. Our assays included 4 dosages for each isolate and 30 beetles per dosage. Beetles were reared individually and observed daily for one week. Each assay was done at least 3 times on different dates for each set of isolates. A standard strain (GHA) was included in each assay. Probit analysis was used to estimate slopes and LC50’s. Average survival times were compared and contrasted. Results showed that recombination between vegetatively compatible strains occurred in vivo at very low frequency. Quantitative changes in pathogenicity or virulence have...
not yet been detected among recombinants. The movement of virulence-related genes during heterokaryon formation has been shown among plant-pathogenic fungi. Further studies are needed to determine if this phenomenon occurs among insect-pathogenic strains of B. bassiana.

Horizontal transmission of Beauveria bassiana between cadavers and adults of Leptinotarsa decemlineata

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Behavior and infection of newly emerged adult Colorado potato beetles (Leptinotarsa decemlineata) in the presence of Beauveria bassiana infected cadavers was studied to determine the likelihood of transmission of disease as beetles emerge from the soil and colonize their host plants. In 2001, arenas were constructed to accommodate reseeded potted greenhouse grown potato plants surrounded with soil to simulate the field environment. B. bassiana killed and sporulated adult beetles were placed in varying patterns surrounding a release point for healthy beetles in the center of the arena. Laboratory reared, newly eclosed beetles were buried just below the soil surface at the release point and were observed for 30 minutes as they emerged and colonized one of four plants. Beetle movements were recorded within a superimposed grid consisting of 5 x 5 cm squares. The study was replicated in 2002 using a similar grid in a potato field. In both the arena and field, emerging beetles showed no preference for movement in any cardinal direction, and direction was not impacted by the presence or absence of B. bassiana sporulating cadavers, nor did the presence of cadavers impact the time taken to colonize a plant or the distance traveled by a beetle. Relative humidity (RH) was a significant factor for both time to colonize and distance traveled to the plant, with longer and lengthier travel times as the RH declined. The plant colonization behavior of newly emerged Colorado potato beetles does not appear to be altered by the presence of B. bassiana in the immediate environment. The likelihood of emerging adults contacting sporulated cadavers on the soil surface was quantified at different cadaver densities.

The impact of scavenging insects on disease persistence in Colorado potato beetle populations

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Previous studies have demonstrated the potential for white-muscarine disease persistence in Colorado potato beetle (CPB) populations due to horizontal transmission of Beauveria bassiana (Deuteromycotina) from conidia produced by primary infected cadavers on the soil surface to soil dwelling stages of the host. Observations in the field indicate that a community of ground-dwelling arthropod scavengers may be responsible for the disappearance of infected cadavers prior to production of conidia and hence, declines in transmission potential. During two summers (2001 and 2002), studies were conducted to characterize the ground-dwelling arthropod community, as well as to establish the rate of cadaver disappearance in three Maine potato fields that differed in insect diversity and abundance of the dominant ground beetle, Harpalus rufipes. Laboratory feeding trials were also conducted (2002) with the predominant species of ground beetles to examine palatability of CPB cadavers of different qualities. Pitfall traps were used to assess the relative scavenger abundances and cadavers were placed in the fields to monitor for disappearance on an hourly or daily basis. Data analyzed for both years show a significantly higher proportion of cadaver decline in the potato fields that contained the highest number of H. rufipes caught per trap per day ($p<0.05$). Correlation between H. rufipes mean relative abundance and the slope of cadaver decline is significant ($p=0.016$). Laboratory feeding trials demonstrate that, although it is primarily a weed seed predator, H. rufipes will consume diseased cadavers.

The intraguild interactions of the greenhouse whitefly predator Dicyphus hesperus with the entomopathogen Beauveria bassiana

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Prey-predator-pathogen interactions are ubiquitous and may play a significant role in population biology and biological control. Under laboratory conditions, we studied intraguild interactions between the heteropteran predator Dicyphus hesperus and the hyphomycete Beauveria bassiana (GHA), two biological control agents of the greenhouse whitefly Trialeurodes vaporariorum. The suitability of infected prey for predator D. hesperus was determined through measurement of the prey acceptance rate as signaled by the consumption of whitefly pupae. Individual D. hesperus predators (second instar or adult females) were presented either one infected whitely treated 1, 2, 3 or 4 days prior, or one uninfected whitely. The incidence of prey feeding indicated by stylet insertion into a whitely pupae was evaluated at two timeframes. Predators were less discriminatory towards recently infected prey in comparison to those where the infection had substantially developed. Within 120 minutes of the first predator-host contact, acceptance was 58.1% lower for nymphs and 23.9% lower for adult females presented prey treated four days earlier in comparison to untreated prey. After 24 hours, prey acceptance was 35.0% lower for nymphs and 27.2% lower for adult predators when presented prey treated four days earlier in comparison to untreated prey. These results indicate that prey acceptance depends on the timing of the infection. Prey rejection was likely linked to visually perceivable changes in the infected prey which could be seen as soon as three days after receiving the B. bassiana treatment. Such changes included the development of extensive hyphal growth and or the acquisition of a red pigmentation due to the presence of oospores produced by B. bassiana. The specific mechanisms by which the predator detects infection in the host are currently being investigated. Understanding of such trophic and guild interactions will contribute to the formulation of effective pest management programs.

Interactions between impatiens pollen, Beauveria bassiana and adult female Western flower thrips (Frankliniella occidentalis)

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Pollens have been demonstrated to increase the fecundity of various species of thrips. Gerrin et al. (1998) has established that populations of the Western flower thrips (WFT), Frankliniella occidentalis, in crops of garden impatiens, Impatiens wallerana, grow at a significantly slower rate when flowers are not present. Previous research conducted by Ugone et al. demonstrated that female thrips strongly prefer impatiens flowers that contain pollen. When using slow acting pathogens like B. bassiana, which can achieve up to 85% mortality under optimal conditions in the laboratory, it becomes essential to know what impatien pollens has on daily and lifetime fecundity as well as longevity of female thrips exposed to B. bassiana. A factorial experiment was conducted to test the effects of pollen versus no pollen in the presence or absence of B. bassiana. Pollen significantly increased lifetime fecundity of female thrips, and exposure to B. bassiana significantly decreases longevity of adult female thrips.

Comparison techniques and parameters used in compatibility tests between Beauveria bassiana and chemical pesticides in vitro

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The procedures used in compatibility studies between chemical products and entomopathogenic fungi are generally very diversified turning difficult, and sometimes almost preventing the comparison of results obtained and published in different articles. The objectives of
this study were to test and to compare the different techniques used in compatibility tests between entomopathogenic fungus Beauveria bassiana and synthetic chemicals in order to provide basic information for the development and establishment of a protocol for in vitro tests. Four modes of contact between the entomopathogenic fungus Beauveria bassiana (CG432), the fungiicide Iprodione (Rovral CS®) and Azoxystrobin (Amistar GR®) and the insecticide Endosulfan (Thiodan CE®) in three dosages each (1/2 CD; CD; 2xCD) that were obtained utilizing the average commercial dosage (CD) for different crops. The techniques consisted in incorporating the chemicals to the culture medium (IM), mix the conidia to a solution of the chemical (MC), and spray the chemical before (SB) and after (SA) inoculation of the fungus into Petri dishes (9 cm 2). The toxic effect of the products was studied through parameters of germination, colony forming units (CFU), vegetative growth (VG) and sporulation (SPO). The inhibitory effect of the fungicide azoxystrobin (CD) on conidia germination and CFU was higher in the IM technique than on the other ones but the effect in SPO was lesser in IM and higher in SA. Also VG was more affected on SA. For iprodion (CD) no difference for GER was observed between techniques but, for CFU in SA and SB the inhibition was significantly lesser. For SPO the higher inhibitory effect was observed for SA. Endosulfan is more toxic for GER, in SA and SB, with high inhibition levels. For CFU the inhibition was higher for IM and MC. VG was more affected in IM and SA techniques and SPO showed more inhibition levels in IM. Results demonstrated that differences among the techniques, for compatibility tests of B. bassiana and pesticides in vitro, do exist thus demanding a standardization of compatibility tests in vitro. Key words: entomopathogens, pesticides, selectivity, standardization.

**STU Poster / Fungi. F-13.**

Effect of growing media and water volume on conidial production of Beauveria bassiana and Metarhizium anisopliae

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Mass production is an important component of a successful microbial insecticide program. The objective of this study was to evaluate conidial production of two isolates of Beauveria bassiana (SPT22 and CA 24) and one Metarhizium anisopliae (500B) using corn, wheat, and millet and three water volumes (substrate: water) (1:0.5), (1:1) and (1:1.5). The results showed that there were significant differences (P<0.007) in conidia production among isolates depending on water volume and growing media used. M. anisopliae produced significantly (P<0.05) more conidia than the B. bassiana isolates. For the B. bassiana isolates, conidial production on wheat was significantly higher (P<0.05) for the 1:1 and 1:1.5 volumes than the 1:0.5 volume. No significant differences were found in conidial production for SPT22 on corn and millet at the volume regimes tested; whereas, for CA 24 there was significantly higher (P<0.05) conidial production for the 1:0.5 volume than the other volumes. Conidial production of M. anisopliae was significantly higher (P<0.05) for the 1:1.5 than 1:0.5 and 1:1 volumes on the three test substrates. These results provide useful information to develop simple efficient mass production techniques for the isolates tested.

Poster / Fungi. F-14.

Molecular characterization and comparative virulence of Beauveria bassiana isolates for control of the shore fly, Scatella stagnalis, on greenhouse crops

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The shore fly, Scatella stagnalis, commonly occurs in large numbers in commercial greenhouses, where it is both a nuisance pest and a vector of several plant pathogens. High density populations can be difficult to suppress with chemicals, and there are no biological control products currently registered for use against S. stagnalis in the United States. A number of reports of natural epizootics of the entomopathogenic fungus Beauveria bassiana in greenhouse populations and laboratory colonies of S. stagnalis suggest it may have potential for biological control of this pest. We conducted a series of studies to assess the diversity of B. bassiana isolates found naturally associated with a colony of shore flies established from a hydroponic lettuce production facility, and to compare these isolates to commercially available B. bassiana products. RAPD-PCR was used to assess genetic variation of B. bassiana isolates from S. stagnalis adults and pupae, and adults of Hexacola neoscutellae, a hymenopteran parasitoid of the shore fly. Sixteen single spore isolates were resolved into three distinct genotypes using 12 primers. The two most common genotypes were found to be similar to ARSEF 252 and 5813, isolated from laboratory colonies of the Colorado potato beetle in Maine and Michigan, respectively. The third genotype was observed in only one isolate, obtained from a S. stagnalis pupa. None of the genotypes were similar to B. bassiana strain GHA, the basis for Botanigard, a mycoinsecticide registered in the U.S. for control of greenhouse pests. Further genetic analyses are planned for B. bassiana isolates obtained from the algal food source of these insects to ascertain whether this could be a natural reservoir for fungal inocula. Additionally, bioassays are currently underway to assess virulence of the three genotypes to all life stages of S. stagnalis and to compare this to that of commercially-available isolates of B. bassiana.


Occurrence of Hyphomycete fungi from natural birch habitats and eroded land in sub-arctic Iceland and Faroe Islands

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In the last decade there has been increasing efforts in afforestation and land reclamation in Iceland and to some extend also in the Faroe Islands. At present the majority of all tree seedlings in both countries are produced in containers, using Sphagnum-based peat as the growth medium, before they are transplanted to disused agricultural land or eroded areas. However, high seedling mortalities are recorded after transplantation. One of the main reasons for this high mortality has been attributed to soil dwelling insect larvae from the genus Otiorhynthus. It has, however, been shown that seedling mortality can be significantly reduced by inoculation of seedlings with soil from old forest stands before transplantation to the field. This indicates that beneficial soil organisms may play a crucial role in plant establishment. This project aimed to study the occurrence of entomopathogenic fungi from 1) natural birch habitats 2) disused agricultural land, 3) eroded areas and 4) sphagnum-based peat from Iceland and the Faroe Islands. Furthermore, selected isolates were characterized by morphology, physiology and molecular methods with focus on their adaptation to the sub-arctic environment.

In our study no entomopathogenic fungi were found in either the peat or in the soil collected from eroded land. In contrast the Beauveria bassiana, Metarhizium anisopliae and Paecilomyces farinosus were documented from natural birch and grass habitats in both countries. Initial screening of selected M. anisopliae isolates from the Faroe Islands and Iceland has shown that they have a higher radial growth rate at lower temperature than isolates of the same species originating from Denmark and Panama. Furthermore, there was a correlation between PCR profile and geographical origin of M. anisopliae isolates from Iceland, Faroe Islands, Denmark and Panama, respectively.

Biological control with native strains of entomopathogenic fungi may thus provide a potential to minimize the damage caused by Otiorhynthus larvae. Parallel studies on birch root symbiotic mycorrhiza status are being carried out and we plan to test the effect of inoculation of M. anisopliae and mycorrhiza on the soil biota under field conditions.
In Danish greenery and Christmas tree plantations two of the most important pests are the nut leaf weevil *Strophosoma melanospermum* and the European field cockchafer *Melolontha melolontha*, causing serious damage on the needles and roots, respectively. At present, no insecticides are approved in Denmark for control of these pests. Biological control including the use of insect pathogenic fungi from Hypomyces may thus provide a potential to minimize the damage caused by these pests. Based on laboratory bioassays one isolate of *Metarhizium anisopliae* and one isolate of *Beauveria brongniartii* were selected to control *S. melanogenum* and *M. melolontha*, respectively, under field conditions. A significant control effect of *M. anisopliae* against *S. melanogenum* was seen, when the fungus was sprayed unto the ground of a Noble fir plantation at 10^8 spores ha^{-1}. The accumulated density of nut leaf weevils in the *M. anisopliae* treated plots was reduced by approx. 50% compared to the control. The effect was not significant until the year after treatment due to a 2-year life cycle of the weevils.

In a Nordmann fir Christmas tree plantation good control effect of *B. brongniartii* on the tree health was observed when the fungus was mixed with soil into the planting hole during planting in a concentration of 30–50 g barley kernels per tree (equiv. to 300–500 kg barley kernels ha^{-1}) The barley kernels (*Meloccont, Agrifutur, Italy*) were colonised with *M. brongniartii* mycelium and the growth and sporulation occurred in the soil. The damage on the trees was estimated from tree health and colour, which were scored in the spring and again in the autumn. Damage was higher in the control plots than in the plots treated with *B. brongniartii*. The roots on some of the trees were also examined for damage and a good correlation was found between root damage and tree vitality score.

Both *M. anisopliae* and *B. brongniartii* have a high potential for controlling *S. melanogenum* and *M. melolontha*, respectively, in greenery and Christmas tree plantations.

**Biological control of weevils and scarabs in greenery and Christmas tree plantations**

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**Characterization of in vitro destruxin production, pathogenicity, and RFLP patterns of peptide synthetase genes in *Metarhizium anisopliae***

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*Metarhizium* species have been at the forefront of efforts to develop entomopathogenic fungi as insect biocontrol agents. Yet we have an incomplete understanding of the biological and genetic factors that make them effective, such as the role of toxins as virulence factors. The principal toxins produced in fermentation by *M. anisopliae* are the destruxins, a large family of cyclic depsipeptides possessing a range of toxic effects. We and others predict that destruxins are synthesized nonribosomally via a large multifunctional enzyme called a peptide synthetase (PS). To date, the destruxin peptide synthetase gene has not been isolated. We cloned DNA fragments encoding putative PS genes from *Metarhizium* spp. and identified restriction fragment length polymorphisms (RFLP) that differentiate *M. anisopliae* strains and closely-related species. We measured destruxin production in *vitro* by 16 strains of *M. anisopliae* and characterized the virulence of 8 strains against beet armyworm. All isolates produced detectable amounts of destruxins A, B, and E in *vitro*, but quantities varied greatly among isolates. Approximately one-third of the isolates produced low (<1 mg/liter), intermediate (5-30 mg/liter) or high amounts (70-170 mg/liter) of destruxins. Greater destruxin production in *vitro* generally correlated with a decrease in insect survival time. However, one isolate that produced low amounts of destruxins in *vitro* killed larvae in the same amount of time as high destruxin producers. This result and similar examples reported in the literature might suggest that destruxins are not required for virulence. An alternative explanation is that in *vitro* production of destruxins does not predict virulence or metabolite production in *vivo*. Our results form the basis for further genetic studies to explore the pathway for destruxin biosynthesis and its relationship to fungal virulence. An understanding of the role of toxins in pathogenicity is essential for enhancing *M. anisopliae* as a biocontrol agent and to confirm its safety against non-target organisms.

**Development of a biologically based pest and disease management system in sugar beets**

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Sugar beets (*Beta vulgaris* L.) are beset by one important insect pest, the sugarbeet root maggot (*Tetanops myopaeformis*), several lesser pests such as wireworms (*Coleoptera: Elateridae*), and a trio of significant diseases, (1) seedling diseases caused by *Aphanomyces* and *Pythium*, (2) Rhizoctonia Crown and Root Rot, and (3) Cerco-spora Leaf Spot. Although sugar beets are grown on 550,600 hectares in the U.S. (2002) they are considered a minor crop and farmers have generally correlated with a decrease in insect survival time. However, one isolate that produced low amounts of destruxins in *vitro* killed larvae in the same amount of time as high destruxin producers. This result and similar examples reported in the literature might suggest that destruxins are not required for virulence. An alternative explanation is that in *vitro* production of destruxins does not predict virulence or metabolite production in *vivo*. Our results form the basis for further genetic studies to explore the pathway for destruxin biosynthesis and its relationship to fungal virulence. An understanding of the role of toxins in pathogenicity is essential for enhancing *M. anisopliae* as a biocontrol agent and to confirm its safety against non-target organisms.

**Detection of strains of *Metarhizium* within infected sugar cane borers, *Diatraea saccharalis*, using specific primers**

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The ITS rDNA have been an important molecular tool for fungi identification. In this study we used the ITS-5.8S rDNA regions were analysed in different species of entomopathogenic fungus *Metarhizium* including *M. anisopliae*, *M. album* and *M. flavoviride* in order to construct specific primers for their detection and identification. Within infected larvae of *Diatreaa saccharalis*. The amplification of these regions yielded an unique fragment of 540 bp approximately for *M. anisopliae* var. *anisopliae*, of 650 bp for *M. album* and 600 bp for *M. flavoviride*. The PCR products were
digested with the different restriction endonucleases *Afa* I, *Alu* I, *Hae* III, *Dde* I, *Hpa* II, *Sau* 3A and the PCR-RFLP profiles showed clear differences among the species. The ITS-5.8S rDNA regions sequencing allowed to construct specific primers for all investigated species. DNA extracted from infected larvae by *M. anisopliae* strains *C* and *E* from Brazil and 14 from Australia in individual bioassays were tested using previously constructed specific primers. In all of them the fungus was detected after 48 hours post-inoculation. This molecular tool will allow to detect infected host as well as the fungus strain more rapidly and efficient in bioassay as well as in pest management programmes.

**Variability in response to heat among strains of *Metarhizium anisopliae* isolated from sites at latitudes from 61°N to 54°S**

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Fungi and other eukaryotic organisms differ markedly from prokaryotes and archaea in tolerance to heat. Fungi, with few exceptions, have limited viability at temperatures above 45°C. We evaluated the tolerance of seventeen entomopathogenic *Metarhizium anisopliae* strains isolated from latitudes 61°N to 54°S. Conidia were suspended in Tween 80 solution (0.01% v/v) and exposed to 40° or 45°C for 2, 4, 8 and 12 h. Relative percentage of germination based on controls levels, was assessed on PDAY medium plus Benomyl 0.002% at 28°C for 48 h. Most of the isolates tolerated 40°C very well, with relative germination above 90% after 12 h of exposure. Exceptions were three strains with relative germination below 80%, which originated from high latitude, viz. ARSEF 2038 (latitude 38°N, South Korea), ARSEF 4295 (54.4°S, Australia) and ARSEF 5626 (61.2°N, Finland). High variability was observed at 45°C after 2 h exposure: six isolates had high relative germination (above 80%), three isolates showed medium tolerance (between 50 and 70% relative germination), and eight isolates had low tolerance (between 0 and 30% relative germination). After 8 and 12 h at 45°C, only the strains isolated from grasshopper (ARSEF 324 and 3609; latitude 19°S, Australia and 15°N, Thailand, respectively) had high relative germination (91.6 and 79.4%, respectively, for 8 h; and 90 and 47.1%, respectively, for 12 h). These isolates also were the most tolerant to UV-B irradiation (Braga et al., 2001. *J. Invertebr. Pathol.* 78, 98-108). The LD50 for the most resilient strain ARSEF 324 was 49.7°C and 48°C, respectively for 2 and 4 h of exposure. In general, isolates from higher latitudes were more heat susceptible than those strains from nearer the equator. Exposure of conidia to heat greatly delayed germination, similar to the findings of Braga et al., 2001 when conidia were exposed to UV-B irradiation. *This research was supported in part by National Council for Scientific and Technological Development (CNPq) of Brazil, supporting PhD fellowship for the first author.*

**Relative performances of fungal pathogens isolated from acridid hosts collected in desert locust habitats in Eastern Ethiopia**

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In this work, we evaluated the performance of twenty-four fungal isolates, obtained from twenty-nine sites from Eritrea, Ethiopia and Somalia. The isolates were tested for their potential to control desert locusts, *Schistocerca gregaria* (Forskal) and migratory locusts, *Locusta migratoria migratorioides* (Reiche & Fairmaire). *M. anisopliae* var. acridum (IMI330189), a highly virulent isolate used as a biopesticide against *Schistocerca gregaria*, was included in all tests as reference standard. Bioassays have been completed on eighteen species, most of which were *Metarhizium* spp. Isolates were tested in soil formulations at low dose (2 x 104 conidia/ml in 2 ul of water (200 conidia/insect)) and at high dose (3 x 105 conidia/ml in 1 ml of water (4,000 conidia/insect)). Control mortality was 5% in both formulations. Most isolates showed differential responses of native target species to *Metarhizium* spp. such that isolates DPV3, DPV5, DPV10, DPV12, DPV14, and DPV15 are highly effective under laboratory conditions against *L. migratoria* and merit testing as oil-based field formulations to differentiate their performance under semi-natural conditions.

**Efficacy of locally collected isolates of *Metarhizium anisopliae* var. acridum and *Metarhizium flavoviride* on three acridid pests in Senegal, West Africa**

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Locusts and grasshoppers are major crop pests in Senegal and in other countries across Sahelian West Africa. Huge quantities of synthetic pesticides are being used to control these pests causing serious environmental concerns. In order to find practical alternatives to this control method, we have embarked in the search for microbial agents that could be pathogenic to acridids and that could be developed as biopesticides. Nineteen locally collected fungal isolates of the genus *Metarhizium* were evaluated for virulence on three important acridid pests in the Sahel: the African migratory locust, *Locusta migratoria migratorioides* (R&F), the bird locust *Orithaecis turbida cavroisi* (F), and the Senegalese grasshopper *Oedaleus senegalensis* (K).

Isolate IMI330189 of *Metarhizium anisopliae* var. *acridum*, developed as a biopesticide by the LUBILOSA project and registered in W. Africa, was used as a check. Two μl of 1.2x107spores/ml aqueous spore concentrate (24,000 spores/insect) were topically applied beneath the pronotum of 3rd to 4th instars of the respective species. Of the fifteen new isolates tested on *L. migratoria*, seven showed a relatively fast killing speed, giving mortality of 28-75% on Day 4 post-inoculation, whereas the check did not exceed 4%. Of six isolates tested on *O. turbida* and three isolates tested on *O. senegalensis*, none performed the check. We have conclude that there were differential responses of native target species to Senegalese isolates of *Metarhizium* spp. such that isolates DPV3, DPV5, DPV10, DPV12, DPV14, and DPV15 are highly effective under laboratory conditions against *L. migratoria* and merit testing as oil-based field formulations to differentiate their performance under semi-natural conditions.

**Influence of submerged cultivation additives and formulation ingredients on the tolerance of blastospores of *Metarhizium anisopliae* var. *acridum* to thermic stress under fluctuating regime**

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Because of severe climate conditions prevailing in areas targeted for locust control, influence of both fungus fermentation and propagation formulation was studied as a means of assessing blastospore quality
of *Metarhizium anisopliae* *var.* *acidum* (isolate IMI 330189 kindly provide to Virginia Tech by CABI Biosciences). Quality evaluation consisted of exposing stabilized propagules to daily fluctuating temperature at 13-43°C under humidity conditions regulated at 13% RH and testing viability through colony growth assays (CFU counts). The survival of Stabilize™-formulated blastospores originated from Jackson media with increasing C/N ratios (10-50) showed a significantly better tolerance to thermic stress of propagules formed in lower C/N ratio conditions. Attempts for optimizing Stabilize™-formulated propagule quality in adding ingredients during the liquid cultivation (at 96h) in Jackson medium demonstrated clearly a negative effect of glycerol alone or in combination with sucrose, Tween and corn oil. The relative loss of viability of blastospores produced with Jackson medium added with Tween and sucrose was similar, over a period of 60-day exposure, to that of blastospores produced in Jackson medium without additives. Formulation assays showed that drying temperature during the dehydration phase of fresh blastospores is a key factor for improving the robustness of dried inocula. Sucrose was tested as additive ingredient in submerged culture as “sugar shock” according to the Stareze™ process, and as formulation ingredient during the harvesting phase of fungal biomass added with hydrated silica (HiSil). In both cases, the addition of sugar did not increase the survival of dried blastospores exposed to temperature stress. Effect of talc, hydrated silica and Kraft lignin (according to a VT- improved procedure) showed a rapid decay of talc-formulated blastospores, a possible effect of the two other formulation ingredients. *Project “Development of Biopesticides for Grasshopper and Locust Control in Sub-Saharan Africa”, funded by the USAID (Africa-Bureau-Funded Project Grant No AOT-G-00-97-00386-00). **Quimby et al., 1996, 1999; Zidack & Quimby, 2001; ***Quimby et al., 2001.*

**Development of potential *Metarhizium* biocontrol agents: insights from molecular data**

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Developing and bringing a mycoinsecticide to market is a multi-tiered process that includes, the identity authentication of the strain, finger-printing development for environmental monitoring and patent registration. The limited potential of conventional strain typing in the hypomycete genus *Metarhizium*, using classical criteria, such as morphological and behavioral characteristics has led to a more systematic genetic assessment of these fungi these past ten years. In this current molecular markers based on the specific characteristics of the West African *Metarhizium* isolates tested and on the type of information necessary to evaluate each particular step in the development process of a biopesticide. The isolates originated from different hosts and geographical areas in Senegal. Sequence analysis of the ribosomal DNA (rDNA) internal transcribed spacer (ITS) allowed the classification of these isolates into the *Metarhizium anisopliae* var *acidum* group. Only at this intraspecific level, ITS and 28S rDNA region analysis detected little or no variation. Genetic relatedness of these isolates was assessed by Random Amplified Polymorphic DNA (RAPD) and subsequently by Amplified Fragment Length Polymorphism (AFLP) analyses. Patterns generated by both methods showed extensive polymorphism and the isolates were easily differentiated. However, no close correspondence has been established between the clustering of these isolates and their host or ecological origins. Ultimately, some of the RAPD markers will be converted to SCAR markers for the production of diagnostic assays which will contribute markedly toward developing a quality control system and would allow post-release monitoring of these commercially important isolates.

**Paeclomyces fumosoroseus against *Trialeurodes vaporariorum* under laboratory conditions**

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Epizootic potential is an important factor in determining the efficacy of an entomopathogen in any spray program. This study introduces a simple bioassay used to determine the epizootic potential of three *Paeclomyces fumosoroseus* Trinidadian strains (*T*, *T10* and *T11*) against *Trialeurodes vaporariorum* fourth-instar nymphs in the laboratory under optimum conditions (25 ± 0.5 °C, ~100% RH). *T. vaporariorum* were arranged on laminated graph paper to simulate varying levels of infestation on a leaf surface, with the central nymph placed in a droplet of *P. fumosoroseus* blastospores suspended in Triton X-100 and the others in equal-sized droplets of distilled water. Percent germination of spores and conidial production on *T. vaporariorum* cadavers were determined prior to the bioassay. The total number of hosts (including exuvia) colonized by the fungus was recorded 7, 14 and 21 days post-treatment during the 24 or 16:8h LD period and was converted to a proportion by dividing by the total number of hosts per grid. The converted data were then used to determine the mean proportion of *T. vaporariorum* hosts ± SEM colonized on the grid
arrangement. Different grid arrangements containing the nymphs at both photoperiods were observed and recorded during the same time period. Statistical analysis using Sheffe F-test showed no significant differences between strains in the production ratios of conidia per cadaver / blastospores ml−1 (F=2.77 = 2.2, P = 0.14). Only the most virulent of the P. fumosoroseus strains as determined in prior tests (T11) was assayed at a very high density. Even in the presence of Cladosporium spp., T11 killed 72% of hosts within 21 days post-treatment. The proportion of hosts colonized at different simulated levels of infestation began to be significantly different 12 days after treatment under a 24h LD photoperiod, and 14 days after treatment under a 16:8h LD photoperiod. The longer the photophase, the greater the percentage of T. vaporariorum hosts or host exuviae colonized, and as the host density decreased, colonization decreased, but not proportionally. This bioassay technique was able to indicate differences in epizootic potential under optimal temperature and humidity conditions at different photoperiods.

**Poster / Fungi. F-27.**

**Collective and individual effects of Paecilomyces fumosoroseus and Encarsia formosa for control of Trialeurodes vaporariorum on beans and Regal geraniums**

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The most efficient control of any pest using biological control agents is achieved when the agents involved in the program are compatible and/or synergistic. In this study, the effect of separate and combined activity of Paecilomyces fumosoroseus Trinidadian strain T11 and Encarsia formosa was assessed on populations of Trialeurodes vaporariorum infesting Phaseolus vulgaris (French bean) and Pelargonium x domesticum (Regal geranium) plants to determine which treatment or combination of treatments most effectively controlled T. vaporariorum. Bean and Regal geranium plants were first infested with T. vaporariorum; however, bean plants were much more heavily infected than T. vaporariorum under identical exposure conditions. Some infested plants were exposed to E. formosa for 24 hours. Four days later, plants were sprayed with 2ml of Triton X-100 only or P. fumosoroseus blastospores suspended in Triton X-100 to runoff on days later, plants were sprayed with 2ml of Triton X-100 only or P. fumosoroseus blastospores. This was repeated using both type of plants that were not infested: French bean (237 ± 35 /mm2) and Regal geranium (218 ± 25 /mm2). 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Field incidence of *Nomuraea rileyi* and evidence that multiple strains are present in the same field

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Natural occurrence of *Nomuraea rileyi* was monitored in soybean pests in Blackville, SC for three years (2000, 2001 and 2002). Velvetbean caterpillar, green cloverworm and soybean looper were the prevalent susceptible noctuid larvae in the field. Infection levels were low in 2000 and reached 2.26% and 0.5% on GCW and VBC, respectively. Gradually increasing levels of infection in soybean fields were observed in 2001 and 2002. Infection in GCW was higher than in other noctuid larvae in 2001. Infection levels for all hosts were higher in 2002, with the first observation of infection in beet armyworm and corn earworm larvae and a significant epizootic in velvetbean caterpillar populations.

VBC was not infected with *N. rileyi* early in the season even though GCW infection levels were high during this period. There was a five-week delay in infection of VBC’s compared to GCW’s. This might be evidence that there were two distinct strains operating in the same field. For this reason, *N. rileyi* isolates from VBC, GCW (early and late season collection), SBL (early and late season collection), FAW and BAW were tested in laboratory bioassays against VBC. The results from bioassays indicated that, VBC was susceptible to *N. rileyi* isolated from VBC with 100% infection at a concentration of 1000 conidia/mm³. However, infection levels were very low (0 to 5%) when *N. rileyi* isolates from GCW, SBL, FAW and BAW were tested against VBC.

**Inhibition of the host immune reaction by entomopathogenic fungus *Nomuraea rileyi***

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The entomopathogenic fungi such as *Nomuraea rileyi* generally inhibit their host immune reaction. To convince the inhibition of host immune reaction to *N. rileyi* (strain SdNr-1: isolated from *Spodoptera depravata*), hemocytos and their phagocytosis in the hemolymph of three species of Noctuidae (*S. depravata, S. litura* and *Mythimna separata*) were observed after conidial injection of *N. rileyi*. The number of plasmatocytes and granular cells were decreased after injection of SdNr-1 to *S. depravata* compared with other lepidopteran larva. In the hemolymph of *S. depravata*, the phagocytosis and encapsulation by these hemocytes to FITC labeled conidia was inhibited by injection of *N. rileyi*. The phenoloxidase activity of hemolymph of *S. depravata* was reduced after injection of *N. rileyi* compared with other two species. Moreover, change of protein in the hemolymph were observed using SDS-PAGE. These results indicated the SdNr-1 had highly pathogenicity to *S. depravata* and the pathogenicity was caused by the effective inhibition of immune reaction of *S. depravata*.

**Analysis of the chitinase gene of the dimorphic mycopathogen, *Nomuraea rileyi***

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A chitinase-encoding gene from *Nomuraea rileyi* consists of an open-reading frame (ORF) of 1271 nucleotides. The ORF contained a 18 aa putative signal sequence with a positively charged region, a hydrophobic domain, and a signal sequence cleavage site at positions 19 and 20 (AL/GLA). Analysis using ProtParam Tool revealed that the 405 aa mature chitinase (w/o signal sequence) had a molecular mass of 43,941 daltons and an aliphatic index of 68.27 and is considered to be a stable protein with an instability index (II) of 26.19. The *N. rileyi* chitinase contains 33 aspartic acids, 9 glutamic acids, 9 arginines, 7 histidines, and no lysines and is acidic protein with a calculated pI of 5.26. Amplification of the genomic DNA with primers designed from the flanking regions of the ORF produced a 1710 bp band. In addition, the genomic material contained three introns, an 111 bp intron at position 264, a 67 bp intron at position 67, and an 60 bp intron at position 412. The deduced amino acid sequence of the mature enzyme exhibited two highly conserved regions of the catalytic domain belonging to the family 18 glycosyl hydrolase. Comparisons of the deduced ORF to other fungal chitinases of family 18 glycosyl hydrolase showed 74%, 71%, and 69 % similarities with *Mortierellaceae flavoviride*, *Aphaenocladium albus*, and *M. anisopliae*, respectively. A dendogram showed that the cloned *Nomuraea* chitinase belongs to the class V fungal chitinase. In addition, the data inferred from the chitinase alignment demonstrated that *Nomuraea* and *Mortierellaceae* are phylogenetically closely related genera.

**Mycopathogens of Homalodisca coagulata, the Glassy-Winged Sharpshooter***

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The glassy-winged sharpshooter (*Homalodisca coagulata*), recently introduced to southern California from the southeastern United States, is a major threat to the grape industry due to its ability to vector *Xylella fastidiosa*, causative agent of Pierce’s disease. In North Florida and South Georgia localized populations of GWSS were found to host fungal epizootics. In this study, we examined a series of mummified cadavers in an effort to identify the causal agent and determine its method of transmission. Several fungi were isolated from cadavers and were examined using a combination of light and electron microscope methods. The dominant fungal isolate produced mononematous conidiophores, elongate echphialdes and lemon-shaped conidia. On Sabouraud maltose + yeast extract agar these isolates produced slow growing colonies having a colony phenotype similar to that of *Hirsutella*. A second group of isolates produced an asporogenous white colony phenotype. Partial sequence analysis of several genes including tubulin and 18S ribosomal DNA were generated from this and placed it close to plant-associated *Mycosphaerella*, *Cappnodium* and *Acremonium*. Likely this later group represents a secondary contaminant. No evidence of horizontal transmission was found when insects collected from areas without the epizootic were confined with mycosexed cadavers in sleeve cage arenas. Collections made of live nymphs and adults from areas with an ongoing epizootic yielded very few insects harboring the pathogen of interest. Based on our observations we speculate that the fungus infecting GWSS, is closely affiliated with the host plant having either an epiphytic or endophytic association.

**Influence of the entomopathogenic fungus, *Verticillium lecanii*, on an aphid parasitoid, *Aphidius colemani*, and a predator, *Chrysopa pallens***

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Aphids are some of the most serious pests in greenhouse vegetables in the world. Several biological control agents have been used as alternative control methods to chemical pesticides. Natural enemies are known to be influenced by pesticides and entomopathogenic microorganisms. Natural enemies and microbial pesticides may be used simultaneously in the field. The possibility of the entomopathogenic fungus *Verticillium lecanii* infecting parasitoids and predators was examined. Cotton aphids were first exposed to the parasitoid *Aphidius colemani* for 24 hours and then sprayed with *V. lecanii* (1x10⁸ conidia/ml) 0, 3, 5, and 7 days after exposure. The mummification of aphids following spray treatments 0 and 3 days after exposure to the parasitoid was 35.9 and 84.8%, respectively, and the...
emergence of the parasitoid was 5.6 and 13.1%, respectively. Infection of larvae and parasitism was confirmed by stereo-microscope and agar medium. Mummification following sporulation treatment 5 and 7 days after exposure to the parasitoid increased to 86.5 and 93.7% and emergence was 79.9 and 91.7%, respectively. No parasitoids were infected by fungus. Treating aphids with fungus 1 hour and 1 day before exposure to A. colemani showed a significant decrease of mummification compared with fungal spray after exposure to the wasp. Therefore, simultaneous application of both A. colemani and V. lecanii needs to consider the developmental stage of parasitoid. Green lacewing (Chrysopa pallens) also is used to control aphids. The larva of green lacewing also was infected by V. lecanii, but the corrected mortality was only 25% 5 days after treatment. Therefore, this result suggests that V. lecanii is relatively harmless to green lacewing larvae.

Poster / Fungi. F-35.

Comparison of Japanese and American isolates of Entomophaga maimaiaga
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Because of the mysterious appearance of the fungus in 1989, many have hypothesized that the E. maimaiaga currently in the U.S. is different from the Japanese biotypes that were introduced in 1910-11 and 1985-86. This hypothesized increase in effectiveness could be from the introduction of a more virulent or pathogenic Japanese isolate or the evolution of one of the isolates previously released in the U.S. This paper examines the variation in pathogenicity and virulence in the two separated populations of fungi (5 isolates from Japan and 5 isolates from the U.S.). We measure virulence of the isolates by injecting protoplasts into 4º instar larvae and measuring the proportion and speed of kill. Pathogenicity will be tested by immersing 4º instar larvae into solutions of fungal conidia collected from larval cadavers. The values of pathogenicity and virulence of the U.S. isolates are not significantly different than the Japanese isolates.

Poster / Fungi. F-36.

Influence of insecticide treatments, irrigation, and BT cotton on population dynamics of the cotton aphid, Aphis gossypii Glover and its pathogenic fungus, Neozygites fresenii (Zygomyctes: Entomophthorales)

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Cotton aphid populations were monitored in 2002 in cotton fields in Blackville, SC, to determine aphid population levels and incidence of infection by Neozygites fresenii. Plots were designed to compare insecticide treatment (Karate) vs. no treatment, irrigation vs. no irrigation, and BT cotton vs. conventional cotton. Counts of live aphids and fungus-killed aphids were made in the field twice weekly. At the same time that counts were made, aphid samples were preserved in alcohol, and later processed in the laboratory to confirm presence of N. fresenii. Aphid populations increased earlier and reached higher levels in plots treated with Karate than in untreated plots. Fungal infection also reached high levels earlier in Karate-treated plots, but epizootic pressures were similar in both treatments. Aphid populations peaked earlier in non-irrigated than in irrigated plots, but the timing and intensity of fungal epizootics were similar in both treatments. Aphid populations were higher in BT cotton (Bollguard) than in conventional cotton plots, but there was no difference in infection levels by N. fresenii and epizootic patterns were similar in both treatments. It appears that various management practices can influence population levels of aphids and cause small differences in timing of N. fresenii epizootics. However, none of the treatments caused disruption or major changes in epizootic patterns.

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Poster / Fungi. F-37.

Do Vicia faba plants use the aphid pathogen Pandora neoaphidis as a bodyguard?

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There are many examples of plants manipulating the behaviour of insect predators and parasitoids to increase their impact on plant damaging herbivores. The plants use the parasitoids and predators as ‘bodyguards’. Unlike insects, there are very few examples of entomopathogenic fungi acting as plant bodyguards. Pandora neoaphidis is an aphid-specific fungal pathogen. Hosts include the pea-aphid Acrystaphthon pismum, which is a herbivore of Vicia faba plants. Here we investigated whether P. neoaphidis could be used by V. faba as a bodyguard against A. pismum.

Infestation of V. faba plants by A. pismum causes the plant to release aphid species-specific volatiles. These volatiles attract the aphid parasitoid Aphidius ervi, which uses them to locate host aphids. Aphidius ervi may therefore be a bodyguard of V. faba, protecting it against A. pismum. If these volatiles have a direct effect on P. neoaphidis, then this fungus could also act as a plant bodyguard. Here we present results from bioassays designed to assess the effect of A. pismum-induced V. faba volatiles on the sporulation rate, conidium size, growth rate and, germination rate of P. neoaphidis.

Initial results indicate no effect of A. pismum-induced plant volatiles on the sporulation rate, growth rate or conidium size of P. neoaphidis. However, exposure to these volatiles did increase the proportion of conidia germinating on aphids compared with exposure to volatiles from undamaged V. faba plants. This could represent a mechanism for P. neoaphidis to act as a plant bodyguard.

Poster / Fungi. F-38.

In vitro interactions between two fungal pathogens of Plutella xylostella: Pandora blunckii and Zoophthora radicans

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Isolates of the entomophthoralean fungi Pandora blunckii and Zoophthora radicans have been collected from diamondback moth, Plutella xylostella, populations. Both species have been reported infecting individuals from the same larval population in the same place. This provides an interesting system to study interspecies interactions, particularly the potential for competition and niche separation. An initial experiment was done to determine whether these species had different temperature optima which could temporally and spatially separate them under field conditions. Thirteen isolates of Z. radicans and 21 of P. blunckii were grown at four different temperatures, 15, 20, 25 and 30°C and their radial growth rates compared. In general, isolates of Z. radicans grew faster at 25°C than P. blunckii isolates and vice versa at 20°C, even though there were some isolates of both species from the same geographic area with similar temperature optima. Based on these results the two fastest and the two slowest isolates of each species at 20 and 25°C were selected for in vitro competition studies. Two colony plugs of fungus, either from the same isolate or different isolates (136 combinations), were placed 2.5 cm apart on plates of nutrient medium. The growth rate of each colony towards each other and the interactions that occurred when the perimeters of the two colonies met were recorded at 20 and 25°C. Results will be discussed in relation to the competitive abilities of each isolate.
The Helicosporidia are obscure pathogenic eukaryotes that have been isolated from various invertebrates. Recently, comparative analyses have shown that these pathogens are non-photosynthetic green algae, and they are related to Protorectum, another non-photosynthetic, parasitic algal genus. In an effort to better characterize the biology of Helicosporidium spp., a cDNA library has been constructed and expressed sequences tags (ESTs) have been generated. To date, a total of 1275 sequences have been obtained. Interestingly, only half (49%) of these clones have significant similarities to genes with a known or predicted function listed in public databases. Many cDNA matched with housekeeping genes and about 75% of them exhibit similarity with algal and plant genes. Several sequences corresponding to the most conserved genes were translated in silico and used in phylogenetic analyses in order to confirm the algal nature of Helicosporidium sp. Additionally, the EST library was found to contain sequences coding for nuclear-encoded, plastid-targeted genes, suggesting that Helicosporidium sp. may have retained a chloroplast-like organelle and an overall plant-like metabolism. Finally, the EST library includes a number of clones that provide insights into the biology of Helicosporidium sp. as a non-photosynthetic alga.

The protist Helicosporidium sp. is an entomopathogenic algae that is characterized by a infectious cyst stage that contains an elongate filamentous cell and 3 ovoid cells. This infectious cyst dehisces within the midgut lumen penetrates column epithelium gaining ingress into the hemocoel. Within the nutrient rich hemolymph this pathogen undergoes multiple cycles of vegetative replication. The resulting in vitro growth leads to production of fully differentiated cysts that are infectious per os to insects. Successive transfers of these cultures results in a decline in cyst production with a concomitant selection of vegetative cell growth. Multiply-passaged cultures are characterized by growth the formation of nonmotile adherent cells that cluster together via intracellular mucilage (palmelloid cell phenotype). Attempts to produce cysts from palmelloid cultures have failed. In vitro we have analyzed the morphogenesis of the different cell phenotypes. In vitro produced cysts partitioned from vegetative cells using Ludox gradients can be readily dehisced using sterilized insect digestive fluid. Released filamentous cells have been purified and observed in vitro. The filamentous cells go through a period of regeneration that is characterized by the thickening of the anterior portion of the filament reorganization of the nuclear material. DAPI staining has revealed nuclear division followed by deposition of daughter cell wall material. The parental filament cell wall eventual ruptures along its horizontal axis and releasing oval-shaped daughter cells. The timetable for this regeneration is as follows: initial 24 hour period results in thickening of the of the anterior filament cell; 24-48 h nuclear division initiated; and by 72 daughter cells are released from filament cell. Daughter cells then elongate and divide into spherical shaped vegetative cells that undergo autosporegeny. Typically the vegetative cells will produce four cells per mother cell. The daughter cells will be released and undergo additional cycles of vegetative growth. After multiple cycles a portion of the vegetative cells differentiate into the specialized cyst stage of Helicosporidium.

The gypsy moth (Lymatrina dispar L.) is known as a serious defoliator of deciduous forests. Dimilin® as a chemical pesticide or Dipel® as a biopesticide are used for control treatments. It is known that Dimilin® affects the arthropod chitin formation, disrupting the development of the cuticle. Therefore, the development of all arthropods, including non-targets, which ingest Dimilin® can be disrupted. Because of these environmental concerns, a variety of studies investigated the influence of Dimilin® on non-target species, e.g., ground beetles, ants, heteroptera, or fungi; several studies were able to show negative effects on these organisms. Microsporidia are effective regulators of insect populations. The spores of microsporidia contain chitin in the endospore layer. Therefore, these natural antagonists of defoliating insects might also be affected by Dimilin®. We performed bioassay studies to investigate the influence of Dimilin® on the microsporidian spore yield and vitality. In a first set of experiments third instar gypsy moth larvae were starved for 24 hours and orally infected with a German Nosema (microsporidia) isolate. Before or after the infection with the Nosema isolate, some larvae were fed Dimilin® in sublethal doses while some larvae were infected with microsporidia only. All larvae were reared on artificial diet. Developmental parameters were recorded every other day. At the end of the observation period or after the death of larvae, the presence and number of spores was determined microscopically. Spores from Dimilin®-fed larvae and larvae fed with artificial diet only were collected and fed to third instar Gypsy Moth larvae in a second set of experiments. We recorded developmental parameters every second day and checked all gypsy moth larvae for the presence of spores.

The mortality increased up to 80% when Dimilin® and Nosema spores were fed to gypsy moth larvae. The microsporidian infection alone did not cause high mortality rates. When Dimilin® was fed to the larvae before the microsporidian infection, the number of produced spores was significantly reduced. When Dimilin® was fed to the larvae 24h or 6 days after the microsporidian infection, the number of produced spores was not significantly reduced. Mature microsporidian spores, which were washed in Dimilin® solution were as infective as spores stored in liquid nitrogen. However, the spores which were produced in larvae, after Dimilin® had been ingested with the diet, were less infective. The experimental infection rate decreased to 48% or 10%, respectively.

Although most of the infected adults contained few spores, 9.8 % (4/41 individuals) of them were heavily infected with the microsporidium. Two isolates from heavily infected adults in the two areas were 11.4 % (5/44 individuals) and 7.1 % (36/508 individuals), respectively. We examined on the occurrence of microsporidiosis in field populations of the tobacco budworm, Helicoverpa armigera in Kyonggi, Korea and Yokohama, Japan. Their field populations were found to be infested with a microsporidiosis. The infection rates of H. armigera adults in the two areas were 11.4 % (5/44 individuals) and 7.1 % (36/508 individuals), respectively. Although most of the infected adults contained few spores, 9.8 % (4/41 individuals) of them were heavily infected with the microsporidium. Two isolates from heavily infected adults from Korea caused systemic infection in H. armigera larvae and exhibited high pathogenicity to larvae. Also, they produced both Nosema- and Thelohania-type spore. The latter stage type was found only in the muscle, while the former was found in haemocyte, nerve, fat body, silk gland, midgut, malpighian tubule, muscle, trachea and gonad. The microsporidium was transmitted to production of extracellular mucilage and observed using Ludox gradients can be readily dehisced using filter sterilized artificial diet.
the next generation through the egg. We conclude that the parasite is a new microsporidian species, *Fairimorpha kyonggii* n. sp., based on the serological test and the DNA sequence. This new microsporidian parasite may be useful for the control of the tobacco budworm in future.

Poster / Microsporidia. MP-3.

**Occurrence of pathogens in bark beetles (Coleoptera, Scolytidae) from Alpine pine (Pinus cembra L.)**

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Log sections and twigs from bark beetle infested *P. cembra*-trap logs were collected in a managed *P. cembra* forest in the Central Alps of Austria (district Lienz, East Tyrol) at three sites in 1800m, 1900m and 1980m altitude. The material was incubated in breeding chambers in the insectary of the Institute at 24°C (± 2°C) and under long day conditions (L:D = 16 : 8). Emerging bark beetles were removed daily, dissected and checked for infections with pathogens under a light microscope.

The absolutely dominant bark beetle species was *Ips amitinus* which emerged from the trap material. In 548 dissected *I. amitinus*, four different pathogen species could be observed—Rhizopoda: Malamaoba scolyti; Sporozoan: Eugregarina: Gregarina typographi, Neogregarina: Mattesia sp. and Microsporida: Chytridiospora typographi. The evidence of Mattesia sp. was (spore size: 15-19 µm x 6,5-7 µm) in *I. amitinus* is reported for the first time as well as the occurrence of Gregarina sp. (96 x 58 µm). This Gregarina sp. was found in relatively high prevalence (18,1 %), while prevalence was much lower for all other pathogen species in this host. Another *Gregarina* sp. was found in *P. conjunctus*, which was the only pathogen in this beetle species. No pathogens were found in all the other bark beetle species.

**STU Poster / Microsporidia. MP-4.**

**Trypanosomatid infections affect male Aquarius remigis body size: Implications for gerrid mating interactions**

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Body size can have significant impacts on mating system dynamics in a population with an assortative mating system. Studies of natural populations of the water strider *Aquarius remigis* (Heteroptera: Gerridae) demonstrate a positive correlation between the body size of mating males and females. Factors that influence body size, such as habitat quality, food availability, and parasitic infection, can have indirect effects on mate choice in water strider populations and thus affect host fitness. Using field observations from three populations, we investigated the prevalence of trypanosomatid parasites in the water strider host, *Aquarius remigis*, and correlated the presence or absence of infection with measures of body size. We show that the presence of trypanosomatid infection has a significant effect on male body size (infected individuals are smaller) but not female body size. We argue that trypanosomatid infection could influence mating dynamics in *Aquarius remigis* populations and thus impact gerrid fitness, population dynamics, and community structure.

Poster / Microsporidia. MP-5.

**An undescribed microsporidium from Lygus hesperus and Lygus lineolaris**

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An undescribed microsporidium was detected in colonies of the tarnished plant bug, *Lygus lineolaris* and the Lygus bug, *Lygus hesperus*. The parasite originated in the *L. lineolaris* colony, and later contaminated the *L. hesperus* colony. The undescribed microsporidium has been tentatively identified as a *Nosema* sp. The microsporidium was found in four tissues; adipose, alimentary tract, Malpighian tubules, and the gonads. We describe morphological characters of the microsporidium by both light and electron microscopy.

**MICROBIAL CONTROL**

Poster / Microbial Control. MC-1.

The USDA-ARS National Biological Control Laboratory: Expectations for the new facility

D.A. Streett

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The USDA-ARS National Biological Control Laboratory (NBCL) will be located at the Jamie Whitten Delta States Research Center in Stoneville, MS. The NBCL will provide an interdisciplinary team of scientists with facilities for basic and applied research developing practical methods of mass propagation, storage, and delivery of beneficial organisms, as well as targeted release strategies for integrated pest management. Only organisms that have been approved by Federal and State officials for use will be propagated and studied in this facility. In addition to the research labs, space is provided for two pilot plants. These pilot plants will be used in cooperation with private organizations to test the practical applications of propagation techniques and to foster commercial production of biological control agents.

Poster / Microbial Control. MC-2.

**Nematodes and entomopathogenic fungi associated with termites**

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As part of a USDA/ARS project on the biological control of *Coptotermes formosanus*, termites were collected in disparate locations (Australia, China, South Africa, Malaysia, Reunion Island, Singapore, Indonesia and mainland France), killed by cooling, placed onto agar plates and inspected daily in quarantine for at least two months for any pathogenic fungi to sporulate, or nematodes or protozoa to emerge. Restriction Fragment Length Polymorphism (RFLP) analysis of the Internal Transcribed Spacer (ITS) region of the ribosomal DNA (rDNA) repeat unit was used to identify two genera of nematodes, *Mesorhabditis* and *Chroniodiopagaster*, found associated with termites from several genera, including *Odontotermes*, *Cryptotermes*, *Postelectrotermes* and *Coptotermes*. The nematodes are being kept in culture on termites and on *Galleria* larvae for use in lab and field studies. Several species of entomopathogenic fungi were isolated from many outwardly healthy termites, including *Paecilomyces farinosus, P. funerosoroseus*, *Beauveria bassiana* and *Metarhizium anisopliae*. Several *Metarhizium* isolates were selected for further study. Sequence analysis of the rDNA ITS allowed the classification of the isolates from Australia, South-East Asia, Reunion and Guadeloupe into the *Metarhizium anisopliae* var.*anisopliae* group only. Relationships within the set of these isolates could not be clearly established using ITS sequence variability; accordingly, they were determined using a random amplified polymorphic DNA (RAPD) analysis. All fungal isolates are being kept in the pathogen collection at the European Biological Control Laboratory, and the utility of some isolates as control agents is being evaluated in field experiments. **Keywords:** termite, nematodes, entomopathogenic fungi, biological control, genetic analysis.
Survey for natural enemies of the alfalfa snout beetle *Otiorynchus ligustici* (L.) in Hungary and in New York State: *Nomia otiorynchii*, entomopathogenic nematodes and entomopathogenic fungi

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Several locations in Hungary and in New York State were surveyed for potential biological control agents of the alfalfa snout beetle *Otiorynchus ligustici* (L.), a serious pest introduced from Europe. The survey focused on entomopathogenic nematodes, entomopathogenic fungi, and the microsporian *Nomia otiorynchii* (Weiser). *N. otiorynchii* was not detected in populations of the alfalfa snout beetle in Hungary. In New York State, a single specimen was found infected with *Nomia*. In Oswego County, a low level of infection was detected in Franklin County. The frequencies of entomopathogenic nematodes and fungi were not significantly different between Hungary and New York State in alfalfa snout beetle infested fields. *Steinerlemma feltiae* was found in Hungary in alfalfa fields but not in New York State. *S. feltiae* was found in coexistence with *S. carpocapsae* in Hungary where the alfalfa snout beetle seemed to be under effective natural biological control.

Poster / Microbial Control. MC-4. Heritability and plasticity of immune function in the Egyptian cotton leafworm

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Phenoloxidase (PO) is believed to be a key mediator of immune function in insects and has been implicated in non-self recognition and in resistance to a variety of parasites and pathogens, including baculoviruses and parasites. Using larvae of the Egyptian cotton leafworm, *Spodoptera littoralis*, we found that, despite its apparent importance, haemolymph PO activity varied markedly between individuals even when reared under apparently identical conditions. Sib-analysis methods were used to determine whether individuals varied genetically in their PO activity, and hence in one aspect of immune function (heritability, $h^2 = 0.69 \pm 0.069, P < 0.001$).

PO activity in the haemolymph was strongly correlated with PO activity in both the cuticle and midgut ($r \geq 0.65, P < 0.05$); the sites of entry for most parasites and pathogens. Haemolymph PO activity was also strongly correlated with the degree to which a synthetic parasite (a small piece of nylon monofilament) was encapsulated and melanized ($r \geq 0.62, P < 0.01$). In addition, levels of PO in the cuticle corresponded to resistance to fungal infection. The mechanism maintaining genetic variation in immune function has yet to be elucidated.

Poster / Microbial Control. MC-5. Evidence for suppression of immunity in honey bees by parasitic *Varroa* mites

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*Varroa* mites are a major contributing factor to recent honey bee loss and have been previously suggested to kill bees by activating bee pathogens. We hypothesize that mites feeding upon bees immunosuppress the bee via salivary protein secretions, in a similar manner as ticks feeding upon mammalian hosts. We also hypothesize that the immunosuppression of *Apis mellifera* by *Varroa* is linked to greater susceptibility of bees to non-specific pathogens and results in bee mortality. We tested this hypothesis by injecting either *Escherichia coli* or saline into bees of known age, mite infestation level, and with or without Deformed Wing Syndrome (DWS). Following challenges with bacteria, bees infested with *Varroa* have lower survivorship than bees without *Varroa*. Bees with a single, domestic colony, bees with deformed wings were always associated with mite infestation and the degree of wing deformity was positively correlated with the number of mites. However, mites did not always cause wing deformity, even when present in high numbers with the developing pupae. Bees with DWS died within 48 hours after eclosion and died even more quickly following bacterial challenge at eclosion. The survivorship of uninfected, healthy bees with normal wings was much longer and was not significantly different between bees with or without *Varroa*. Following bacterial challenge, the mite-infested bees with normal wings died significantly faster than normal, healthy bees without *Varroa*. There was no relationship between the number of mites and level of immunosuppression. Evidence for a period of immunoimmunity was found in newly eclosed honey bees since phenoloxidase (PO) activity was not detected within the first 24 hours in newly eclosed bees that were challenged with immunodeficiency. We hypothesize that the bees with deformed-wings die due to activation of a latent pathogen by mite feeding, coupled with this period of immunocompetence at adult eclosion. Various immune components and responses were tested for impairment by mite infestation. Hemocyte numbers and quality were significantly different among bees with and without *Varroa*. Hemocytes from bees with DWS have putative viral particles. Despite the lower hemocyte numbers, bees with DWS and with mite infestation mounted a strong encapsulation response. PO activity is elevated in bees with mite infestation at 36 hours as compared to bees without mites. Bees with mites have significantly less FAD-glucose dehydrogenase activity as compared to bees without mites, suggesting that the killing response during cellular immunity could be impaired. Salivary proteins from *Varroa* have been isolated for proteomic analysis using 2D gel electrophoresis and mass spectroscopy.

Poster / Microbial Control. MC-6. Microbial control of the Colorado potato beetle in irrigated desert: combinations and alternations of *Bacillus thuringiensis* and *Beauveria bassiana*

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In the Pacific Northwest of the USA, insect control in potatoes is focused on the green peach aphid due to its importance in the transmission of potato leaf roll virus. Colorado potato beetle is controlled incidentally by systemic insecticides, such as aldicarb, directed at the aphid. These systemic insecticides may be unavailable in the near future because of impending regulatory actions, requiring development of control strategies for the beetle. Microbial agents offer safe and selective means of control for several insect pests including CPB with minimal effect on nontarget organisms. The efficacy of *Beauveria bassiana* and *Bacillus thuringiensis* as microbial control agents of CPB has been well documented, however, the majority of the research has been conducted in areas where humidity remains fairly high during the growing season. Relatively little research on microbes for control of CPB has been carried out in irrigated desert. In studies conducted in three consecutive seasons we investigated the effect of combining and alternating the *B. bassiana* and *B. thuringiensis* on feeding by beetle larvae and their effect on yield. Four weekly applications of *B. thuringiensis* and a mixture of *B. thuringiensis* and *B. bassiana* were made in experimental plots to determine effects on densities of CPB, defoliation, and yield in 1998-2000. Unsprayed and aldicarb-treated plots were included as experimental controls. For both defoliation and yield in 1998, the mixture of both microbial agents substantially outperformed plots that received *B. thuringiensis* alone. Field trials of two microbial control agents (individually and mixed), transgenic potato (Newleaf), and aldicarb for control of CPB and their effects on nontarget organisms were conducted in 1999. Effective control of CPB populations was observed following just two applications of *B. thuringiensis* or mixtures of *B. thuringiensis* and *B. bassiana*. In 2000 similar levels of control were obtained with *B. thuringiensis* and with the mixture of half label rates of *B. thuringiensis* and *B. bassiana* throughout most of the growing season. The treatments had a significant effect on numbers of living overwintering beetles. The mixture of these two pathogens appears to provide an alternative to systemic insecticides for managing CPB in central Washington.
Assessing environmental risks of biological control agents: a general framework
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In more than 6000 biological control attempts exotic natural enemies have been imported, mass reared and released during the past 100 years. Negative environmental effects of these releases have rarely been reported. To ensure the continuing safety and positive public image of biological control, many countries are requiring risk assessment for biological control agents. A methodology has been developed within the European Union financed project “ERBIC” as a basis for regulation of import and release of exotic natural enemies used in inundative biological control. In this presentation I will explain the general framework of a risk assessment methodology for biological control agents, integrating information on the potential of an agent to establish, its abilities to disperse, its host range, and its direct and indirect effects on non-targets. The parameter ‘host range’ forms a central element in the whole process, because lack of host specificity might lead to unacceptable risk if the agent establishes and disperses widely, whereas, in contrast, a monophagous biological control agent is not expected to create serious risk even when it establishes and disperses well. To illustrate the method, the proposed risk assessment methodology is applied to a number of biological control agents currently in use, including the fungi Beauveria bassiana and Metarhizium anisopliae, and the nematode Steinernema feltiae. Among wide variety of currently used biocontrol agents such as parasitoids and predators, these agents ranked in this initial exercise in the middle range as being ‘moderately safe’. These case histories indicate that the risk assessment methodology can discriminate between agents, with some species attaining low ‘risk indices’ and others scoring moderate or high. Risk indices should, however, not be seen as absolute values, but as indicators to which a judgement can be connected by biological control experts for granting permission to release or not.

Acknowledgements: This study was partially funded by the EU-projects ERBIC (EU-FAIR5-CT-1997-3489) and MASTER (EU-QLK5-CT-2001-01447). http://www.rothamsted.bbsrc.ac.uk/pic/master/master.htm

NEMATODES
Poster / Nematodes. N-1.
Entomopathogenic nematode delivery systems for biological control of pests on major outdoor crops: the case of oilseed rape
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Timing, dose, and method of application were considered in experiments conducted to explore the potential of Steinernema feltiae for the control of oilseed rape pests Meligethes aeneus and Phyllotreta spp. A field test was carried out using optimum timing (beginning of pupation of M. aeneus, early July) and a ‘sufficient’ dose (1 million IJ/sqm), applied with watering can. The treatment was extremely efficient against the most important rapeseed pest in Finland, the pollen beetle: 93.8% control. Flea beetles were reduced by 50.1% despite the suboptimal timing of application. Concerning non-target effects, the overall numbers of macro-Diptera were slightly affected (-17%), while ‘Hymenoptera parasitica (166%), spiders (+19%), and ‘micro-Diptera’ (+3%) were affected positively or not at all. However, the pollen beetle specific parasitoid Phradis moriontelus was dramatically reduced in the following spring, by 94.4%, equaling the reduction of its host. Problems in translating these results into practice include: (1) it is not possible for a farmer to treat at the optimum time (end of flowering), and (2) the high dose is prohibitively expensive. To overcome these, we constructed a low dose, slow-release nematode delivery system (’NemaBag’). In 2002 a field test on oilseed rape was conducted with 6 treatments, applied 1 week after sowing (end of May): control, S. feltiae spray at 450 kI/sqm, S. feltiae in NemaBags at the rates of 450, 150 (2 dates), and 15 kI/sqm. The results were inconsistent. Three of the treatments reduced the flea beetles similar to results from the previous year, but now obtained with much lower dose of nematodes. None of the treatments, however, had a significant impact on pollen beetle numbers, unlike the results from previous year. The modest impact in this experiment may be due to hostile external conditions at the time of application, as well as the conducive soil type available (high clay content). These results indicate that one can get equal results from treating the soil with a high dose (450 000 IJ/sqm) in water solution, and from applying far less nematodes in a slow-release system such as the NemaBag at a rate as low as 15 000 IJ/sqm. Even with the lower rate it was possible to establish nematodes in a plot, capable of killing larvae 3 months after application. We conclude that EPN show good activity against key oilseed rape pests, and that the slow release, low rate delivery system merits intensified study as a possible way towards practical use of EPN in major outdoor crops.

Acknowledgement: This study was partially funded by the EU-project MASTER (EU-QLK5-CT-2001-01447).
http://www.rothamsted.bbsrc.ac.uk/pic/master/master.htm

Conservation of entomopathogenic nematode populations through the manipulation of crop diversity in vegetable production systems
Janet Lawrence, Casey Hoy and Parwinder Grewal
Dept. of Entomology, Ohio Agricultural Research and Development Center, The Ohio State Univ., Wooster, Ohio 44691

Diverse vegetable landscapes are plagued by a complex of pests, many of which have the potential to be managed by entomopathogenic nematodes, Heterorhabditis spp and Steinernema spp. Use of these beneficial nematodes within vegetable cropping systems has been explored through inundative releases which although sometimes successful, have been uneconomical as repeated releases are necessary. Developing strategies to sustain the efficacy of inundative releases throughout a cropping season would assist in improving the economics of using these nematodes. Typically, heterogeneous vegetable crop assemblages are characterized as having a diverse array of insects (pests and non-pests). Previous studies have shown that nematode persistence is related in part to the abundance and composition of hosts; therefore, increasing plant diversity within fields should increase the availability of hosts in which nematodes can recycle and persist. Plots consisting of mixtures of oats, clover, turnips, parsley as well as pure oats, and bare ground were established in a complete randomized design with four replicates in the vegetable production area of Northeast Ohio. Heterorhabditis bacteriophora (HP88 commercial source) and H. megidis (endemic strain from Northeast Ohio) were applied to all plots at a density of 10,000 infective juveniles/m2. Arthropod and nematode populations were assessed at three-week-intervals. Results are discussed in the context of manipulating plant communities to conserve and enhance nematodes within vegetable cropping systems.

Poster / Nematodes. N-3.
Dispersal of entomopathogenic nematodes incorporated into a stochastic and spatially explicit model of population dynamics
Casey W. Hoy, Janet Lawrence, and Parwinder Grewal
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A mathematical model of nematode population dynamics was extended to explore the impact of nematode dispersal on population dynamics. The ultimate goal of the project is conservation of entomopathogenic nematode populations in annual cropping systems through habitat management. A simple descriptive model was used to simulate H. bacteriophora population dynamics in 1 m2 patches of soil. Spatially explicit, stochastic simulations of multiple patches were then used to reflect the heterogeneous environment in the field. Results have been used to guide research into the nematode population dynamics that can explain survey results in the field. The current project extended the model to include patches representing a cultivated field, patches representing a grassy field border, and dispersal of nematodes across the border between the cultivated field and the grassy border. Important parameters for measurement in field

– 60 –
Low Cost Liquid Fermentation of Entomopathogenic Nematodes

Naomi Pye1, Edgard Carvalho1, Wayne Curtis2, and Albert Pye1

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Entomopathogenic nematodes are used as biological control agents for certain pest insects. Other potential applications are limited by production volume and cost. Large-scale production in traditional bioreactors has only modestly impacted production costs due to the expenses associated with conventional bioreactor design and operation. In this work we used a recently described “Curtis” low capital investment bioreactor with Steinernema felifae and its symbiotic bacterium to gain the economies of scale provided by liquid culture while retaining flexibility in production to meet seasonal demand. These initial experiments used a 10-liter prototype to investigate pH, color and other easily measured parameters useful to quantitatively, but inexpensively, monitor the growth and interactions of symbiotic bacterium and nematode. We concluded that 1) A major function of the symbiotic bacterium is to condition or digest food, not just to grow and have its cells be the food.2) Optimal conditioning appears to correlate with an increased pH and a color change about 36 hours after bacterial addition, 24 hours after peak bacterial concentrations were observed. And 3) The Curtis bioreactor gave yields of infective juveniles (IJ’s) comparable to those of standard reactors (i.e. >60,000 IJ’s/ml) with nematode inoculum of about 5000 IJ’s/ml.

Field survey and evaluation of entomopathogenic nematodes for white grub Phytophaga vetula (Horn) control in Oaxaca, Mexico

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To isolate entomopathogenic nematodes, during Summer-fall 1998 and 1999, a total of 446 soil samples were collected in eight natural regions of Oaxaca State, México. The Cañada Region, an irrigated area, showed the largest percent of positive samples (8.9 %) followed by the Northern Sierra Region (8.1 %), and the Southern Sierra region (5.7 %) and Tuxtepe (2.7 %). Drought prone areas such as Valles Centrales and Mixtcoa had 2.1 and 2.3 % positive samples, respectively. Very hot and dry regions such as Costa and Istmo did not give positive samples. The largest percentages of positive samples were obtained in medium textured soils, with adequate soil moisture including irrigated or temperate zones. Three promising isolates were obtained, two from Cañada (Sample 25 and Sample 2), y another one from Sierra sur (Sample 10). Those were identified as Steinernema felifae Filepjev, Steinernema sp., and Heterorhabditis sp., respectively. Lethal mean dosages ranged from 87 to 105 nematodes per white grub for Steinerneuma carpocapsae Weiser (ALL strain) and S. glaseri Steiner (NC strain), to 146-263 nematodes per larva for the promising isolates. However, lethal dosages to control 95 % of the population ranged from 369 to 3910 nematodes/larva, the highest corresponding to Isolates No. 10 and No. 2. The relatively high LD95’s associated to local nematodes may imply a low pathogenicity of these isolates. Therefore, to find more aggressive species, it is advisable to carry out more surveys in the future, especially in temperate and irrigated areas.

Evasive behavior of white grub species against entomopathogenic nematodes

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Emphasis on biological alternatives to pesticides has increased in agriculture due to concern about environmental pollution. Entomopathogenic nematodes (EPNs) are used as biological control agents for soil dwelling insects with varying success. Some grub species, for example, have been shown to vary in susceptibility to EPNs. We hypothesized that differences in the defensive and evasive behaviors of grub species at least partially account for variation in the susceptibility of grubs to nematodes. In this study, we evaluated the evasive behavior of Rhizotrogus majalis, Popillia japonica, and Cyclocephala borealis against Steinernema glaseri and H. zealandica in glass chambers containing field soil. Grub movement was tracked after the inoculation of 2000 infective juveniles in close proximity to the grub for 2 hours. Water was inoculated as a control treatment. Mean distance traveled per 20-min increment, total mean distance traveled in 2 hours, and percent grub mortality, was quantified for each treatment. Third instar Rhizotrogus majalis exposed to S. glaseri moved further away from the inoculation points in 2 hours (mean distance = 43mm) than those exposed to the nematodes (mean distance = 32mm). This experiment will be repeated and detailed results will be presented.
duction. After the nematode invades the insect hemocoel and before an extensive cellular immune response by the insect, the symbiotic bacterium must be released from the nematode gut and established for nematode reproduction. The underlying mechanisms used by the nematode to evade the insect immune response and the countermeasures used by the insect present a unique system for study of host/pathogen coevolution and for discovery of key regulators of cellular immunity. Evidence is presented for the following: 1) the initial, immediate insect immune response is critical, 2) the nematode itself is contributing to overcoming this defense, and 3) H. bacteriophora and S. glaseri elicit a different immune response that correlates with host susceptibility. The interactions of the nematodes and the hemocytes from a series of resistant and susceptible hosts (Manduca sexta, Galleria mellonella, Popilia japonica, and Acheta domestica) were visualized by light microscopy in sterile, in vitro cultures, and captured with time-lapse computer-generated movies (available through a web-site access). In addition, the cellular interactions were examined using scanning electron microscopy. Initial recognition of the nematode by the hemocytes determines success of nematode. For H. bacteriophora (Oswego), the hemocytes of the resistant host M. sexta rapidly recognize the nematode ends and encapsulate the entire nematode, while producing reactive oxygen species. In a semi-permissive host (P. japonica), recognition is also rapid but directed first at the middle of the nematode and then the ends, permitting release of the bacteria. In the susceptible host G. mellonella, hemocyte recognition is weak, allowing release of the bacteria and survival of the nematode. Preliminary data suggest that less than 15 major proteins are present in the surface coat proteins of H. bacteriophora, and that these can disrupt melanization and coagulation by Manduca hemocytes. The nematode/bacterium produce factors eliminating reactive oxygen species that underlie the killing of invaders by insect hemocytes. In a semi-permissive host like the Japanese beetle, these factors may permit the nematode to survive until the bacterium can act. Thus in the case of H. bacteriophora, a triad of interactions governs the fate of the nematode/bacterium versus insect. For S. glaseri, hemocytes from M. sexta fail to recognize the nematode, suggesting that the surface coat proteins are different from H. bacteriophora. A strong immune reaction is elicited by the hemocytes of P. japonica but this response is overcome by the nematode and are known to be mediated by surface coat proteins. In collaboration with Randy Gaugler, Elizabeth Cowles, and Richard Cowles, we are investigating the underlying mechanisms.

BACTERIA – 3

**CONTRIBUTED PAPERS. Monday 2:00-3:45.**

### STU

**Pore-forming properties of Bacillus thuringiensis insecticidal toxin Cry9Ca mutants in the insect midgut brush border membrane**

Jean-Frédéric Brunet1,2, Vincent Vachon1,2, Greta Arnaut1, Jeroen Van Riel2, Jean-Louis Schwartz1,2, Raynald Laprade1,2

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Once ingested by target insects, most lepidopteran-specific Bacillus thuringiensis insecticidal crystal proteins are transformed into active toxins of about 65 kDa by intestinal proteases. The active form is composed of three domains of which domain I, a bundle of seven amphiphatic [α]-helices, is responsible for the toxin’s insertion into the luminal membrane of midgut epithelial cells. This creates a pore that abolishes transmembrane ionic gradients and leads to cell lysis. In the presence of midgut juice, Cry9Ca is subject to further hydrolysis producing an inactive 55-kDa protein. Previous studies have shown that this degradation can be eliminated by replacing the arginine residue at position 164, located in the [β]-[α]-[β] loop of domain I, by an alanine. Other mutations in the interhelical loops of domain I cause modifications in the protoxin activation kinetics and in the pore-forming ability of the activated toxins, as determined by an osmotic swelling assay on brush border membrane vesicles isolated from Manduca sexta. Wild-type Cry9Ca and two of its single-site mutants, R164A and R164K, formed larger pores and showed a weaker ionic selectivity than previously studied toxins of the Cry1 family. Activity of the three toxins was highest at pH 6.5 and declined gradually as pH was increased. Poor activity at pH 10.5 could not be explained by a pH-dependent change in the ionic selectivity of the pores. The rate at which these toxins form pores in membrane vesicles was extremely low, even at pH 7.5, compared to that observed with other B. thuringiensis toxins such as Cry1Ac. Nevertheless, Cry9Ca and its mutants could depolarize the luminal membrane of freshly isolated M. sexta midguts, albeit weakly, at pH 10.5 in a medium of high ionic strength comparable to that found in the midgut. In the presence of midgut juice, however, all three toxins depolarized the midgut membrane, although Cry9Ca and R164K were more efficient than R164A. These results indicate that the pore-forming activity of Cry9Ca is strongly dependent on the physico-chemical conditions under which it is measured. They also suggest that midgut proteases and/or emulsifying agents could play an important role in Cry9Ca activity in the insect midgut.

**STU** Contributed paper. Monday, 2:15

**Differential effects of pH and ionic strength on the pore-forming activity of Bacillus thuringiensis toxins**

Mélanie Fortier1,2, Martin Kirouac1,2, Vincent Vachon1,2, Olivier Peyronnet1,2, Jean-Louis Schwartz1,2, Raynald Laprade1,2

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Bacillus thuringiensis insecticidal toxins act by forming pores in the midgut apical membrane of susceptible insects after binding to specific receptors located at the surface of this membrane. 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 membrane surface and therefore influence the interaction of the toxins with the membrane. Earlier studies have indicated that, in the absence of membrane potential and at low ionic strength, Cry1C forms pores more efficiently at pH 7.5 than at pH 10.5 in midgut brush border membrane vesicles isolated from Manduca sexta. In contrast, the activity of Cry1A remains high over this pH range (Tran et al., 2001, Appl. Environ. Microbiol. 67:4488-4494). The combined effects of toxin concentration, pH and ionic strength on the pore-forming activity of Cry1A and Cry1C were further studied using a brush border membrane vesicle osmotic swelling assay and membrane potential measurements in isolated midguts of M. sexta. In brush border membrane vesicles, increasing ionic strength decreased the rate of pore formation by Cry1A, at pH 7.5 and pH 10.5, and that of Cry1C at pH 7.5, but increased the rate of pore formation by Cry1C at pH 10.5. In isolated midguts, the depolarizing activity of Cry1C, at 10 mg/ml, was comparable to that of Cry1A except at high pH and very low ionic strength. At pH 10.5, increasing ionic strength decreased the activity of Cry1A but increased that of Cry1C. These electrophysiological results correlate well with those obtained with the osmotic swelling assay. The pore-forming ability of Cry1Aa, Cry1Ab, Cry1B and Cry1E was also tested in isolated midguts at pH 10.5 and high ionic strength. At 10 mg/ml, all toxins except Cry1B, which is not toxic to M. sexta, depolarized the membrane equally well. At 1 mg/ml, however, Cry1C was much more active than Cry1Ab, while Cry1Aa, Cry1Ac and Cry1E had little or no activity. Because Cry1C is significantly less toxic to M. sexta than Cry1Aa, Cry1Ab and Cry1Ac, toxin activity appears to be modulated, not only by pH and ionic strength, but also by other factors, possibly including midgut proteases and membrane potential.
Mutations in domain I interhelical loops affect the rate of pore formation by the Bacillus thuringiensis Cry1Aa toxin in insect midgut membrane vesicles

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Pore formation in the apical membrane of the midgut epithelial cells of susceptible insects constitutes a key step in the mode of action of Bacillus thuringiensis insecticidal toxins. In order to study the mechanism of toxin insertion into the membrane, at least one residue in each interhelical loop of the Cry1Aa pore-forming domain (domain I) was replaced individually, using site-directed mutagenesis, by a cysteine, an amino acid which is not found in the activated Cry1Aa toxin. The ability of each of the activated mutants to permeabilize midgut brush border membrane vesicles isolated from the tobacco hornworm, Manduca sexta, was examined with an osmotic swelling assay. Following a one-hour preincubation with the vesicles at pH 7.5, all mutants except V150C (4-5 loop) were able to form pores, although W182C (5-6 loop) had a weaker activity than the other toxins. Increasing pH to 10.5, a procedure which introduces a negative charge on the thiol group of the cysteine residues, caused a significant reduction in the pore-forming ability of most mutants without affecting that of Cry1Aa or T122C (3-4 loop). At pH 7.5, the rate of pore formation was significantly lower for F50C (1-2 loop), Q151C and Y153C (4-5 loop), W182C (5-6 loop) and S252C (7-8 loop) than for Cry1Aa. At pH 10.5, all mutants formed pores significantly more slowly than Cry1Aa, except T122C and I88C, on the other hand, was significantly faster. These results suggest that domain I interhelical loop residues play an important role in the conformational changes leading to toxin insertion and pore formation.

Contributed paper. Monday, 2:30

Role of β-helix 4 in the Bacillus thuringiensis Cry1Aa toxin: cysteine scanning mutagenesis

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Bacillus thuringiensis insecticidal crystal toxins exert their main toxic effect by forming pores in the midgut epithelial cells of susceptible insect larvae. Previous studies on the Cry1Aa toxin have indicated that helix 4 plays an important role in pore formation and lines the lumen of the pores. To further investigate the role of this helix, each of its residues was replaced individually by a cysteine, an amino acid which is otherwise absent from the activated Cry1Aa molecule. The effect of the mutations on the rate of formation and properties of the pores formed by each of these mutants was examined with a light scattering assay and brush border membrane vesicles isolated from Manduca sexta. Pore-forming ability varied considerably depending on the position of the mutated residue within the helix. Most mutations with little or only relatively minor effects on toxin activity map on the hydrophobic face of the helix while most of those causing substantial or complete loss of activity map on its hydrophilic face. Changes in membrane permeability caused by the active mutants, relative to those observed with wild-type Cry1Aa, followed a similar pattern when assessed by measuring membrane permeability to either potassium chloride, N-methyl-D-glucamine hydrochloride, potassium gluconate, sucrose or raffinose. This observation indicates that the main effect of the mutations was a reduced ability to form pores rather than substantial alterations in pore size and ionic selectivity. In agreement with this interpretation, mutants with reduced pore-forming ability also displayed reduced rates of pore formation. On the other hand, pore formation by wild-type Cry1Aa was efficiently inhibited by the presence of a ten-fold excess of either one of the inactive mutants. These toxins therefore appear to bind to Cry1Aa-specific membrane receptors despite their inability to form pores in the membrane. In addition to providing necessary background information for the ongoing characterization of these mutant toxins using thiol-specific reagents, the results of the present study contribute additional evidence that helix 4 plays an essential role in pore formation, particularly at the level of post-binding events probably including toxin oligomerization and insertion into the membrane.

Contributed paper. Monday, 3:00

Cyt1Ca—a new Bacillus thuringiensis subsp. israelensis gene: cloning, purification and characterization of the encoded toxin

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Mosquito larvicidal activity of Bacillus thuringiensis subsp. israelensis (Bti) is included in the following 5 major polypeptides of the parasporal crystalline body (d-endotoxin) produced during sporulation: Cry4Aa, Cry4Ba, Cry10Aa, Cry11Aa and Cyt1Aa (of 134, 128, 78, 72 and 27 kDa, respectively), encoded by the respective genes. The cytotoxic and hemolytic Cyt1Aa, which is not homologous to any of the Cry’s, is most prominent but less specific.

An unknown gene encoding ca. 60 kDa protein has recently been discovered in Bti, named cyt1Ca concordant with the conventional B. thuringiensis toxin nomenclature. Cyt1Ca represents a two-domain fusion toxin: its N-terminal half resembles Cyt1Ab from B. thuringiensis subsp. medellini (52% of 226 amino acids), while its C-terminal half is similar to several toxins containing ricin B-like domain. Cyt1Ca was PCR-amplified from pBtoxis and cloned in several vectors allowing high expression in Escherichia coli. Cyt1Ca was purified by Ni-NTA affinity chromatography as a fusion protein with 6xHistidine residues.

Several biological activities of Cyt1Ca were tested including hemolysis of sheep erythrocytes, toxicity against Aedes aegypti larvae and synergism with different Cry toxins of Bti against mosquito larvae.

Contributed paper. Monday, 3:15

Molecular genetic analysis and enhancement of Cry19A synthesis in Bacillus thuringiensis

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Cry19A is a 65-kDa mosquitocidal protein encoded by orf1 of a two-gene operon in Bacillus thuringiensis subsp. jegathesan. This protein is of potential importance in managing mosquito resistance because it shows only a low level of cross-resistance to other mosquitocidal Cry proteins. Little is known, however, about Cry19A synthesis and crystallization, and the effect that the 60-kDa protein encoded by cry19A operon orf2 has on these processes. Moreover, strategies for enhancing Cry19A synthesis to improve efficacy have not been examined. Here, we show that orf2 is required for efficient net synthesis of Cry19A, and that the 60-kDa protein it encodes apparently acts by stabilizing Cry19A and assisting its crystallization. Cells expressing the wild type cry19A operon produced a low level of Cry19A, and small crystals variable in shape that averaged 0.5 mm in width. Crystal size and thus the amount of Cry19A was enhanced fourfold when expression of the cry19A operon was placed under control of cyt1A promoters in combination with the 5’ STAB-SD sequence that stabilizes transcripts. However, expression of cry19A
alone using cyt1Ap/STAB, yielded only a low level of Cry19A and no observable crystals. The larger crystals produced by expressing the cry19A operon using cyt1Ap/STAB had a shape similar to classic Cry1 bipyramidal crystals, and contained both Cry19A and the 60-kDa ORF2 protein. These results suggest the 60-kDa protein stabilized nascent Cry19A molecules and facilitated their crystallization, functioning like the c-terminal half of 135-kDa Cry proteins. The toxicity of Cry19A cells produced using the cyt1Ap/STAB cry19A operon construct was fourfold greater (LC50 = 1.9 mg/ml) than that of preparations produced using the wild type operon (LC50 = 8.2 mg/ml) against larvae of Culex quinquefasciatus.

Contributed paper. Monday, 3:30

Cyt1A synergizes toxicity of Bs Bin by enhancing its insertion through the mosquito midgut microvillar membrane

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The Cyt1A protein of Bacillus thuringiensis synergizes the toxicity of mosquito cidal Cry proteins and can delay and suppress resistance to these endotoxins. In addition, when combined with the Bacillus sphaericus binary (Bin) toxin, Cyt1A suppresses resistance to B. sphaericus and extends its spectrum of activity to mosquitoes normally insensitive to this bacterium. The mechanism underlying these important properties is not known. Using purified toxins labeled with fluorescent dyes, here we show that Cyt1A restores Bin toxicity to resistant mosquitoes and extends its species spectrum by enabling this toxin to insert into and through the mosquito midgut microvillar membrane. In sensitive larvae, Bin toxin exhibited intratissue specificity, whether alone or in combination with Cyt1A, binding preferentially to cells in the gastric caeca and posterior stomach. Against larvae of Culex quinquefasciatus highly resistant to Bin, or larvae of Aedes aegypti, a species normally insensitive to B. sphaericus, Bin did not bind to any region of the midgut epithelium. However, Bin bound along the entire midgut epithelium when fed with or after Cyt1A.

SYMPOSIUM (Cross-Divisional). Monday, 4:30–6:40

Host altered behavior: Host mediated or pathogen induced

Symposium. Monday, 4:30

Changes in host behaviour: host altered or pathogen induced?

Helen E. Rov

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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 be endeavouring to maximise their 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 only on aspects of host physiology. Changes in host behaviour have largely been neglected. The literature that does exist in this area mainly refers to macroparasites. However, research emerging on pathogen-induced/host mediated behavioural changes demonstrates the range of altered behaviours exhibited by invertebrates including behaviourally induced fever, elevation seeking, reduced or increased activity, reduced response to semiochemicals and so on. In many cases it is difficult to predict whether host altered behaviour is beneficial to the host or to the pathogen. Indeed, a behavioural change may enhance pathogen transmission or host defence in one relationship but not in another. The situation is complex, but it is undoubtedly the case that host behaviour affects fundamental aspects (virulence, propagation, transmission) of the biology of pathogens and ultimately their evolution.

This presentation will aim to introduce and address the question of whether changes in host behaviour are host altered or pathogen induced.

Symposium. Monday, 4:40

Manipulation of host behavior by entomopathogenic fungi

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Fungal pathogens are well known to cause changes in host behavior and, in turn, numerous hosts change their behavior to prevent or cure fungal infections. To prevent infection, some insects orient away from fungal inoculum in the soil or groom themselves to remove fungal spores prior to penetration. Once fungus has penetrated the cuticle, infected hosts often display altered behaviors such as changes in feeding, reproduction, defensive locomotion, and response to alarm pheromones. Locusts and flies have been shown to raise their body temperatures by basking, thus curing themselves of infections when basking occurs soon after fungal penetration. Aphids infected by Pandora neoaphidis wander off host plants before dying, suggesting protection for sisters who then might not be showered by conidia after host death. Perhaps most interesting is "summit disease", the climbing behavior of numerous species of infected insects that results in host death at elevated locations. Fungi are generally thought to benefit when insects die in elevated locations in specific postures, sometimes affixed by the fungus to substrates so that spores are distributed more widely. Studies with Entomophaga maimaiga infecting gypsy moth larvae demonstrate that early instar cadavers producing conidia are attached to tree branches in the canopy, while cadavers producing resting spores are attached to tree trunks. In both instances, the location of cadavers is similar to locations where those instars are normally found, which results in greater chance for aerial dispersal of conidia from early instars and deposition of resting spores at bases of trees where late instars walk. The trick with resting spore cadavers is that death must occur early in the morning when gypsy moth larvae are migrating from the canopy to the leaf litter where they rest all day. Similarly, infected aphids and flies are known to die at precise times of day. Through what physiological mechanisms do fungal pathogens alter host behavior? Infection biases neural circuits that direct normal behaviors, i.e., climbing or walking are coordinated, but in a specific direction over which the insect has no control during the later stages of infection. Current hypotheses differentiate whether such neural effects are mediated globally, by circulating neurohormones, such as biogenic amines that are known to alter the direction of locomotion in some arthropods, or more locally as particular regions of neuropil are penetrated by fungal hyphae. In some infected insects climbing is known to be guided geotactically, but whether the infection simply biases sensory input or is acting in more central neuropil is unstudied.

Symposium. Monday, 4:59

Host manipulation by insect baculoviruses

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Pathogens can manipulate the behaviour, physiology and morphology of their hosts in a variety of ways to enhance their fitness. Some of the earliest descriptions of baculovirus disease mention behavioural changes in infected larvae, in particular, the tendency for infected caterpillars to move up the plant to die, so-called tree-top disease or ‘wipfelkrankheit’. It is assumed this behaviour has evolved to enhance the transmission of the virus that can spread down the plant with either gravity or rainfall. However, although the observed behavioural changes appear to be beneficial for the virus, conclusively demonstrating their adaptive value is more difficult. Baculoviruses can also manipulate their hosts in other, more subtle, ways. The function of many baculovirus genes is still not known, but it evident from those with an ascribed function that not all genes are associated with activities crucial for virus replication or structure: auxiliary genes. Several of these genes have been shown to work at an organismal level. In particular, the edestyroid UDP-glucosyltransferase (egt) gene, influences host moultng, which in turn influences

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the rate at which the baculovirus kills its host. Thus the egt gene appears to manipulate host development, thereby increasing available resources and the quantity of viral progeny produced. As virus productivity is a crucial component of virus fitness, increases in yield should be highly beneficial to the virus.

Symposium. Monday, 5:18

Alteration of host physiology and mating behavior resulting from virus replication

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In order to determine if Hz-2V replication resulting in the malformation of reproductive tissues alters the mating behavior of virus-infected female moths, flight tunnel and mating experiments using infected females and normal male moths were conducted. These experiments revealed that virus-infected females did exhibit calling behavior, and attracted more male moths than did normal females. Pheromone levels in virus-infected females were found to be 6 to 7 times higher than in normal females which, helps explain the increased attractiveness of these females for males compared controls. In mating experiments, normal males attempt to mate with virus infected females however, these are limited to many brief, frequent contacts between mating pairs. This virus mediated alterations in host behaviour as well as the altered mating behaviour observed in experiments involving normal females and virus infected males are thought to play a role in virus transmission and the evolution of virus virulence.

Symposium. Monday, 5:37

Manipulation of sexual reproduction by the intracellular bacteria Wolbachia

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Intracellular bacteria of the genus Wolbachia are among the most abundant endosymbionts on the planet, occurring in at least two major animal phyla—the Arthropoda and Nematoda. Unlike traditional intracellular bacteria of arthropods that often establish highly specific mutualistic associations within a narrow range of host species, Wolbachia parasitize the reproductive strategies of a wide variety of arthropod hosts by inducing parthenogenesis, male-killing, feminization, or a sperm-egg incompatibility termed cytoplasmic incompatibility. Each of these reproductive alterations impart a fitness advantage to infected females, the transmitting sex for Wolbachia, and thereby allow the bacteria to rapidly spread through host populations. These effects can also profoundly influence the evolution and ecology of the infected hosts by altering basic processes such as sex determination, sexual selection, behavior, and speciation. The abundance, widespread distribution, and phenotypic effects of Wolbachia make this intracellular bacterium perhaps one of the most common infectious parasites on the planet and a formidable player in invertebrate evolution. These aspects and further details on the biology of Wolbachia will be discussed in this presentation.

Symposium. Monday, 5:56

Disease resistance in crowds, density-dependent prophylaxis in the Egyptian armymworm

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Several insect species display altered phenology and behaviour during outbreaks, a phenomenon known as density dependent phase polymorphism. The crowded phase is typically triggered by tactile stimulation and in many locusts, phasmid and Lepidopteran species it is characterised by darkening or melanization of the cuticle, making these individuals more conspicuous than solitary phase individuals. The adaptive value of melanin in the crowded phase is unclear but it may be linked to disease resistance. The density-dependent prophylaxis hypothesis, states that as the risk of parasitism and infectious disease is expected to increase with population density, so species that encounter large fluctuations in density may alter their investment in costly immune defences to match the probability of exposure.

Despite growing evidence that insects in high-density populations show increased resistance to certain pathogens, few studies have examined any underlying alteration in immune function. The aim of this study was to quantify relative variation in the allocation of resources to immunity associated with solitary and crowded phases in a phase-polyphenic Lepidopteran species (Spodoptera littoralis). Relative to pale individuals, melanic (typical crowded phase) larvae exhibited higher haemolymph and cuticular phenoloxidase (PO) activity, and exhibited a stronger melanotic encapsulation response to an artificial parasite inserted into the haemocoel. Conversely, pale larvae had higher antibacterial activity than melanic larvae. These results are examined in relation to pathogen resistance, and the possibility of a trade-off within the immune system is discussed.

Symposium. Monday, 5:65

Behavior of nematode-infected insects and of scavengers to nematode-killed insects

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Aberrant behavior of insects infected with nematodes is common. Bumble bee queens infected with a sphaerulariid nematode never establish a nest but fly close to the ground, dig small holes in the soil, and repeat the process a few meters away. These queens are depositing infective nematodes into the soil. In another example, adult female face flies seek proteins for ovarian development from cattle faces. Flies with mature eggs go to cattle dung to oviposit and return to cattle faces for more protein. Face flies, infected with an allantnomatid nematode, change their behavior. Female flies on dung have a higher percentage of nematode infection than those on the faces of cattle. The infected female flies from dung are physiologically older with ovaries packed with nematodes that are deposited onto dung, whereas those from faces of cattle have immature ovaries with young nematodes in the hemocoel. The older infected flies become terminal dung seekers and are less likely to pester cattle. This behavioral change is induced by the nematodes continually invading the ovaries resulting in the flies responding to the need to “oviposit.” Recently, we studied a nematode that is sexually transmitted from male crickets to female crickets. The promiscuous female crickets never become infected with the nematode but serve as vectors to transfer the nematodes to other males during subsequent mating. In a different system, ants are normally scavengers of insect cadavers, but insects killed by the Steinernema-Xenorhabdus or Heterorhabditis-Photorhabdus complex are not scavenged by ants. The ability of Xenorhabdus nematophilus and Photobacterium luminescens to produce an ant deterrent factor(s) (ADF) was tested in vivo and in vitro. ADF activity was present in the supernatants of bacterial cultures, but the amount of ADF repellency depended on the ant species, the sucrose concentration (in vitro assays) or the bacterial strain that killed the insect (in vivo assays). ADF is filterable, heat-stable, and acid sensitive, is eluted through a 10-kDa cut-off membrane, and appears to be a non-proteinaceous compound(s). We conclude that the symbiotic bacteria of some entomopathogenic nematode species produce a compound(s) that deters scavengers such as ants and thus protects nematodes from being eaten during reproduction within insect cadavers.
Prevalence of eukaryotic gut parasites in Drosophila along an urban gradient

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Prevalence is often invoked as an indicator of stress in a host species, with the assumption that environmental conditions alter host susceptibility to infection. Increased prevalence in an insect population may therefore indicate increased stress on these and other animals in a given habitat. If so, then prevalence in common and easily surveyed insects like Drosophila could be an important tool in assessing animal response to habitat degradation, such as that associated with urbanization. However, urbanized habitats are not necessarily “stressful” to animals, and may be, instead, resource rich. To test whether prevalence varies with habitat urbanization, we conducted a six-month survey of eukaryotic gut parasites (trypanosomes and an intracellular fungus) in the community of Drosophila found along an urban gradient in SW Ohio. To provide an independent assessment of habitat quality along this gradient, we also measured host body size: many previous studies have shown that Drosophila in poor habitats are smaller than those reared in optimal conditions. We discuss the correlations between prevalence, host size and environmental variables at six sites along the gradient.

Contributed paper. Monday, 4:45.

Bethylid parasitoids of grain beetles are vectors and potential reservoirs of Mattiesia oryzaephil

Jeffrey Lord
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The neogregarine, Mattiesia oryzaephil, is pathogenic for several stored-grain pest insects, including the sawtoothed grain beetle, Oryzaephilus surinamensis and the rusty grain beetle, Cryptolestes ferrugineus. It also infects their respective bethylid parasitoids, Cephalonomia tarsalis and Cephalonomia waterstoni. Male wasps do not attack the beetle larvae and do not become infected, but the disease is transmitted per os to nearly all female wasps when they paralyze or feed on infected hosts. The mean survival time of diseased C. tarsalis after exposure to heavily infected O. surinamensis was 20 d. For C. waterstoni that were infected via C. ferrugineus, the mean survival time was 36.1 d, as opposed to 45.9 d for uninfected C. waterstoni. The long survival time of infected wasps allows for parasitoid oviposition and transmission. The wasps oviposit on beetle larvae that have early stage infections, and their progeny succumb to the infection. They do not oviposit on beetle larvae with late stage infections that are macroscopically visible under ultraviolet illumination. Living C. waterstoni and to a lesser extent C. tarsalis transmit the disease to beetle larvae as well as serving as a source of inoculum after death.

Contributed paper. Monday, 5:00.

Action of Malamaeba scolyti Purrini (Rhizopoda, Amoebidae) in different bark beetle hosts (Coleoptera, Scolytidae)

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The effects of Malamaeba scolyti was tested in Dryocosmocto autographus, Tomicus piniperda, Hylurgops palliatus, Hylastes ater, Polygraphus poligraphus, Pityogenes chalcographus, Pityogenes calcaratus, Ips typographus, Ips sexdentatus, Ips larisic. Fresh Malamaeba scolyti cysts were yielded from infected midguts of adult Dryocosmocto autographus. Beetles were artificially infected by offering either cysts in water (through drinking) or by offering contaminated spruce phloem chips (through eating) for 24 hours. Afterwards beetles obtained fresh phloem chips (with regard to their food preference, spruce or pine chips) and remained in vessels at 20°C temperature, 92% relative humidity and without light. Beetles were dissected, the midgut and the Malpighian tubules were checked post infection after different incubation periods.

All bark beetle species tested were found to be sensitive to Malamaeba scolyti infections in the laboratory. Infection rates were very different depending on beetle species, number of cysts offered in the drinking water or on phloem chips, even type of water (tap water or A. dest.) had some influence on infection success.

In vitro development of Helicosporidium

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The protist Helicosporidium sp. is a entomopathogenic algae that is characterized by a infectious cyst stage that contains an elongate filamentous cell and 3 ovoid cells. This infectious cyst dehiscs within the midgut lumen penetrates column epithelium gaining ingress into the hemocoel. Within the nutrient rich hemolymph this pathogen undergoes multiple cycles of vegetative replication. The resulting in vivo cells can be harvested and cultured in vitro. This in vitro growth is characterized by the production of vegetational cells that undergo a 2-4 cell asporogenous division. Cell division and daughter cell wall formation occurs within the mother cell. Initial in vitro growth leads to production of fully differentiated cysts that are infectious per os to insects. Successive transfers of these cultures results in a decline in cyst production with a concomitant selection of vegetational cell growth. Multiply-passaged cultures are characterized by growth the formation of nonmotile adherent cells that cluster together via production of extracellular mucilage (palmelloid cell phenotype). Attempts to produce cysts from palmelloid cultures have failed. In vitro we have analyzed the morphogenesis of the different cell phenotypes. In vitro produced cysts partitioned from vegetative cells using Ludox gradients can be readily dehisced using filter sterilized insect digestive fluid. Released filamentous cells have been purified and observed in vitro. The filamentous cells go through a period of regeneration that is characterized by the thickening of the anterior portion of the filament reorganization of the nuclear material. DAPI staining has revealed nuclear division followed by deposition of daughter cell wall material. The parental filament cell wall eventual ruptures along its horizontal axis and releasing oval-shaped daughter cells. The time table for this regeneration is as follows: initial 24 hour period results in thickening of the of the anterior filament cell; 24-48 h nuclear division initiated; and by 72 daughter cells are released from filament cell. Daughter cells then elongate and divide into spherical shaped vegetative cells that undergo autosporation. Typically the vegetative cells will produce four cells per mother cell. The daughter cells will be released and undergo additional cycles of vegetative growth. After multiple cycles a portion of the vegetative cells differentiate into the specified cyst stage of Helicosporidium.

Influence of Helicosporidium spp. infection on development and survival of three noctuid species

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A Helicosporidium spp. (Chlorophyta: Trebouxiophyceae) isolate, recently purified from an aquatic weevil, Cyrtobagous salviniae (Coleoptera: Curculionidae), was capable of infecting and reproducing in three heterologous hosts, Helicoverpa zea, Spodoptera exigua, and Trichoplusia ni (Lepidoptera: Noctuidae). Regardless of host species, oral treatment of third instars with Helicosporidium cysts resulted in about 50% infection of the challenged larvae. The sex ratio did not differ between infected and control groups, suggesting the existence of a natural, non-sex related resistance to the disease. Mating experiments with resistant individuals will enable us to follow this hypothesis in subsequent infection trials with the F1
Previous recombination experiments involving the SalI-F region of the Cydia pomonella granulovirus (CpGV) suggested that it contained a putative origin of replication. This region contains an additional 2.45 kbp of DNA in the Mexican strain of CpGV compared to the Russian strain. An imperfect 76 bp palindrome (rep-7) occurs close to the site of the additional 2.45 kbp. In total, 13 similar imperfect palindromes have been located as singletons within the CpGV genome (Luque et al., 2001). An infection-dependent replication assay was adapted for a Cydia pomonella cell line using an MOI of 0.1. The DNA extracted from cells harvested at 5 d.p.i. was used in DpnI replication assays. The SalI-F fragment replicated in the CpGV-dependent DNA replication assay. A subclone of SalI-F containing a 120 bp fragment incorporating the imperfect palindrome (rep-7) was also able to replicate. Deletion analysis of this fragment and of the other 12 imperfect palindromes is in progress. The CpGV genome also contains one region within the fragment PstI-F, which shows characteristics similar to the non-homologous regions of nucleopolyhedroviruses which have been reported to act as origins of DNA replication (non-hr). However, this region of CpGV did not replicate in the CpGV-dependent DNA replication assay. Studies are underway to characterise all of the putative origins of replication in the CpGV genome.

RNA interference (RNAi) is a cellular mechanism capable of suppressing gene expression that has been detected in many organisms. RNAi refers to the induction of RNAi, which is an antisense RNA molecule that is used to silence the expression of specific genes. This can be achieved by introducing a double-stranded RNA (dsRNA) homologous to the gene to be suppressed. Few such studies have been done on the lepidopteran cells, Spodoptera frugiperda (Sf9). In this work Sf9 cells have been used to express the marker gene for the enhanced green fluorescent protein (EGFP) either transiently, by transfecting cells with plasmids containing this gene, or via infection with recombinant baculoviruses expressing EGFP under the polyhedrin promoter. Additionally, the EGFP gene has been tested either as a single gene, or fused to a scorpion, Leiurus quinquestriatus quinquestriatus, insect toxin (LqIT2) gene, or after the LqIT2 gene but separated by an internal ribosomal entry site (IRES) sequence. The inducers in this gene silencing system were EGFP dsRNA, a small interference RNA (siRNA) consisting of 22 bp from the EGFP coding region, and the EGFP antisense RNA. Also a transformed Sf9 cell line constitutively expressing two copies of the EGFP gene in opposite orientations, to produce an inverted repeat that would form a dsRNA, was established to suppress EGFP expression. The transient expression of the EGFP gene was totally suppressed when the dsRNA and siRNA were the inducers of the RNA interference mechanism. Sf9 cells infected with recombinant baculoviruses expressing EGFP were capable of partially suppressing the expression with either of the inducers. This could be due to the strong promoter expressing the EGFP gene. The stably transformed cells were also found to partially suppress gene expression.

Lipid rafts are lipid-ordered regions within the plasma membrane that contain high concentrations of cholesterol and sphingolipids. These regions are resistant to disruption with non-ionic detergents, like Triton X-100, and exhibit a buoyant nature on flotation assays. There are increasing evidence that lipid rafts play a crucial role in the assembly of enveloped viruses, where viral components are initially concentrated in localised areas of the plasma membrane via their association with lipid rafts. In order to determine whether lipid rafts play a role in the budding of baculovirus virions, Spodoptera frugiperda (Sf9) cells were treated with fluorochrome-conjugated cholera toxin B subunit and examined by confocal microscopy. The random punctate appearance of the fluorochrome within the plasma membrane was consistent with the results from studies in mammalian cells for the identification of lipid rafts. In Autographa californiae nucleopolyhedrovirus (AcNPV)-infected Sf9 cells, the distribution of the fluorochrome was punctate but polarised to discrete regions of the plasma membrane. In co-labelling studies using an antibody to the AcNPV major surface glycoprotein, gp64, the protein was shown to co-localise within lipid rafts at 24 hours post-infection. This result was confirmed by flotation assays in which lipid rafts containing gp64 were isolated from the top of Optiprep density gradients. Control experiments have also been performed in which AcNPV-infected Sf9 cells were treated with methyl-b-cyclodextrin or saponin, cholesterol removal and sequestering agents respectively to disrupt lipid raft formation. Our data suggest that lipid rafts may play an important role during the baculovirus budding process.

Lipid rafts contain high concentrations of cholesterol and sphingolipids. These regions are resistant to disruption with non-ionic detergents, like Triton X-100, and exhibit a buoyant nature on flotation assays.
and E1delE2del infected cells. The E2 peptide antisera detected a protein of approximately 90 kDa in extracts from wild type and E1del infected Ld652Y cells late in infection, but not from E2del and E1delE2del infected cells. E1 and E2 were found, through Western analysis, in preparations of polyhedra and occluded virus (ODV), and were further localized to ODV. E1 and E2 were not found in budded virus preparations. ODV was treated with several detergents that would disrupt the envelope but not the nucleocapsid to determine whether the enhancer proteins are part of the envelope, nucleocapsid, or both. Treatment of ODV with DOC, CHAPS, OGL and NP-40 caused E2 to move from the pellet fractions containing nucleocapsids to the supernatant fractions indicating that E2 is a component of the envelope. Treatment of ODV with DOC caused movement of E1 from the pellet fraction to the supernatant fraction. However, treatment with NP-40, CHAPS, and OGL caused movement of very little of E1 to the supernatant fraction. These results suggest that E1 and E2 may differ in their locations within the envelope and that E1 may be associated with or a component of nucleocapsids. Immunoelectron microscopy revealed that E1 was primarily associated with or a component of ODV nucleocapsids. In contrast, E2 was primarily located at the edge of ODV envelopes. Deletion of the E1 gene resulted in E2 being found in association with ODV nucleocapsids as well as at the edge of ODV envelopes. Deletion of the E2 gene resulted in E1 being found at the edge of ODV envelopes as well as in association with nucleocapsids.

Contributed paper. Monday, 5:30

The immediate early 0 protein IE0 of the Autographa californica nucleopolyhedrovirus is not essential for viral replication

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The AcMNPV protein IE1, product of the immediate-early gene ie1, plays crucial role in regulating the viral infection while the role of IE0 is still obscure. Recently we reported that recombinant AcMNPVs that expressed ie0 at extremely low levels were able to replicate efficiently in poorly permissive S. littoralis SL2 cells in contrast to AcMNPV (Lu et al., J. Virol. 77:535, 2003). This suggested that AcMNPV mutants null in ie0 could be viable and even might be able to replicate efficiently in SL2 cells. To study the properties of an AcMNPV recombinant that does not bear ie0, we constructed vAc[ie0] in which the cat gene replaced exon 0 using targeted mutagenesis. We found that indeed vAc[ie0] replicated efficiently in SL2 cells but the viral life cycle was delayed in SF9 cells. Our results prove that ie0 is not essential for AcMNPV replication, however it is an auxiliary gene that accelerates it and suggest that IE0 may help the virus to overcome the host defense.

Contributed paper. Monday, 5:45

Transcriptional regulation of a Chilo iridescens virus early and late gene

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Chilo iridescens virus (CIV or IV-6) is the type species of the Genus Iridovirus (Family: Iridoviridae). The viral DNA has been entirely sequenced and is about 121 kb in size. To study the transcriptional regulation of CIV, promoter sequences of the DNA polymerase (DNApol) and major capsid protein (MCP) gene were used as a model for an early and a late gene, respectively. Infection of Bombyx mori Bm-36 cells in the presence of Ara-C (inhibits DNA replication) or cycloheximide (inhibits protein synthesis), followed by RT-PCR on isolated total RNA, showed that DNApol is expressed as an immediate-early gene and confirmed that MCP is a late gene. 5'MAP analysis on RNA isolated from CIV-infected Bm cells showed that transcription initiated at position –35 for DNApol and position –15/16 for MCP, relative to the translational start sites of these genes. To determine the limits of the putative promoters, up-stream sequences of various lengths were cloned in front of a firefly luciferase reporter gene. The resulting plasmid constructs were tested in a transfection assay, in which the baculovirus IE-1 promoter fused to Renilla luciferase was used as an internal control for transfection efficiency. Both the DNApol and MCP promoter were only active when cells were simultaneously infected with CIV. The MCP promoter activity was strongly reduced when the length of the sequence upstream of the translational start site was reduced from 67 to 43 nucleotides. For DNApol, the promoter activity was reduced to almost zero when the upstream fragment was reduced from 62 to 41 nucleotides. Changing the G to a C in a TGGTTT motif just upstream of the transcription initiation site of DNApol reduced the promoter activity with 25%. This is the first report of a functional study on insect iridovirus transcription.

Contributed paper. Monday, 6:00

The mechanism of Ha-Vp39 binding to actin and the influence on proliferation and assembly of progeny virions

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Shortly after nucleocapsid of Heliothis armigera nuclear polyhedrosis virus penetration into the cytoplasm, actin cable structure was formed, which associated with nucleocapsids prior to viral gene expression and concomitant with transport to the nucleus. In this paper we report purified nucleocapsid protein Ha-Vp39 can bind to purified actin directly in vitro without helping of assistant factors that was detected by overlay assay and ITC (isothermal titration calorimeter) assay. Meanwhile according to A and binding constants, there is a strong suggestion that Ha-Vp39 bind to actin which has three binding site and acted as core or seeds for actin aggregation to form the cable structure. Cytochalasin D (CD) can inhibit actin to form the cable structure showed the pointed end of actin filaments is uppermost binding site. However actin cable structure is necessary for nucleocapsids transport which is also necessary for viral proliferation, what is the relation between actin and progeny virions? The proliferation of many kinds of nucleopolyhedroviruses (NPV) in cell cultures has been decreased by CD. In this study, we discovered that the proliferation of HaNPV in Hz-AM1 cells grown in the medium containing 0.5 µg/ml CD was completely inhibited while its yield was reduced to 10% in the presence of 0.1 µg/ml CD. However, Western blotting revealed that the actin concentration in infected host cells treated or untreated with CD was almost identical. Additionally, CD had no effect on the DNA synthesis of HaNPV. However, the virions assembled in the CD treated cells were apparently different from normal cells. The incomplete virions resulted in the proliferation of non-infected progeny. The incomplete HaNPV virions are different from the AcMNPV physical particles lacking nucleocapsid formation at 69 cells grown in the medium containing 0.5 µg/ml CD. It is concluded that actin is necessary for nucapsid’s transportation and HaNPV’s successful assembly.

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

Genomics of entomopathogenic nematode-bacterium complexes

András Fodor

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The developmental events resulting in infective juveniles (IJ) of entomopathogenic nematodes (EPN, belonging to the Steinernema...

TUESDAY - 29 July
and Heterorhabditis genera are very similar to those of the dauer larva formation in Caenorhabditis elegans. The genetic regulation of the dauer formation and recovery has been described in detail. Some genes (such as daf-2, daf-16) involved in the process are conserved across animal phylogeny and playing key role in aging and fat metabolism. There are several powerful tools of molecular genetics and functional genomics elaborated by C. elegans research community which might be applied to EPN species even if complete nucleotide sequence of their DNA has not been determined. It is a realistic option, since the genes of similar function are clustered in distinct, multi-megabase regions of individual chromosomes and tend to share translational profile. The similar arrangements of genes of the chromosomes (synteny) in Phylum Nematoda is rather possible although has not been studied so far. The degree of synteny as well as the functional similarities of C. elegans and entomopathogenic nematodes might be determined by RNA interference (RNAi). By this technique the expression of 16,757 of the 19,427 predicted genes of C. elegans (~86%) could be interrupted. The question is what portion of EPN genes could be inactivated by dsRNA copy of homologues genes. In spite of methodological problems the application of the available C. elegans RNAi library of 16,757 bacterial clones to Heterorhabditis and Steinernema may provide information of the similarities and dissimilarities of clustering and expression pattern of genes of similar function. We are suggesting a double-line strategy of introducing RNAi technology into the EPN research: (i) “carpet bombing” strategy, by which some questions concerning synteny could be answered and a (ii) “precision targeted bomb” strategy, by which the functionally identical might be inactivated. From application aspects the identification and inactivation of genes of dauer constitutive mutant phenotypes would be the most important. The second part of the project would be to analyze those megabase-size pieces of EPN the genome which is not related to C. elegans but common in both steinernematids and heterorhabditids. If these segments be found, they should be analyzed for genes playing a role in regulation symbiosis and pathogenicity.

Symposium. Tuesday, 8:35.

Revealing the stress tolerance mechanisms in entomopathogenic nematodes: a genomic approach

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1Dept. Nematology, 2Dept. Genomics and Bioinformatics, Volcani Center, Bet Dagan 50250

The natural habitat for entomopathogenic nematodes (EPN), the soil is a difficult environment for persistence of any organism considering its complexity of physical, chemical and biological components. Nevertheless, EPN have been isolated from soils throughout the world in ecosystems ranging from sub-arctic to arid and temperate to tropical climates. Despite the vast progress in the studies on EPN efficacy and persistence in the soil little is known about the mechanisms of survival.

We used the EPN Steinernema feltiae IS6 as target nematode to study the molecular basis for tolerance mechanisms to heat, desiccation and osmotic-pressure stresses. We utilized advance genomic and bioinformatics approaches. Using cDNA subtractive hybridization we identified IS6 genes that are differentially expressed during exposure to desiccation stress. One hundred and ten genes were identified, among them Late-Embryogenic-Abundant gene (Sf-LEA) and aldehyde dehydrogenase (Sf-ALDH), both are known to be involved in response to water stress in other organisms. Furthermore, using real-time PCR we detected a significant increment in the steady state level of the genes transcription products upon 8 hours of nematodes exposure to desiccation, and further increase upon 24 hours of desiccation. Future studies of desiccation tolerance, including identification of additional desiccation-related genes and study of their biological roles and regulation, will shed light on the genetic and biochemical alterations evolved in environmental-stress tolerant organisms.

Symposium. Tuesday, 9:00.

Sticking and swarming in Xenorhabdus nematophila

Steven Forsl, Hongjun He and Dong-jin Kim
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Xenorhabdus nematophila, a gram negative bacterium belonging to the Proteus clade of the Enterobacteriaceae family, forms a mutualistic association with the soil nematode, Steinernema carpocapsae. The nematode invades insects and releases Xenorhabdus into the hemolymph where it participates in insect killing. To better understand the interaction between the bacterium and nematode, the mrx operon of X. nematophila, which encodes fimbrial appendages that facilitate adhesion to biotic surfaces, was studied. The mrx operon contained 5 structural genes (mrxACDGH) but unlike the mpr operon of Proteus mirabilis, lacked a site-specific recombinease and a mpr-like gene. MxA fimbriae were produced at high levels in cells grown on agar while the Mrp fimbriae in Proteus are not produced on agar surfaces. Thus, the regulation and genetic organization of the mrx operon was found to be distinctive in several respects. Competition experiments showed that a strain lacking the MxA fimbriae could colonize but was not efficiently released from the nematode. Xenorhabdus also displays swarming behavior on agar surfaces. Since the regulatory protein, OmpR, controls flagella production and swarm cell differentiation in several enteric bacteria, an ompR-minus strain of Xenorhabdus nematophila was created. Swarming behavior in the ompR strain began 4 hours sooner than in wild type cells. Precocious swarming in the ompR strain was correlated with early flagellation and cell elongation indicating that OmpR was involved in the temporal regulation of these processes in X. nematophila. The ompR strain also showed a competitive defect in the release of the bacteria from the nematode. Taken together, these results suggest that MxA fimbriae and OmpR play a role in the interaction between the bacteria and the nematode.

Symposium. Tuesday, 9:25.

Negotiating mutualism between Xenorhabdus nematophila and Steinernema carpocapsae

Heidi Goodrich-Blair, E.C. Martens, K. Heumgens, C.E. Cowles, and E.J. Vivas
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The bacterium Xenorhabdus nematophila colonizes the intestine of a non-feeding stage of Steinernema carpocapsae nematodes. The nematode is the vector that carries X. nematophila into insect hosts, which are killed to obtain nutrients for development and reproduction. In adapting to this specialized life style, X. nematophila has evolved functions necessary to be both a symbiont, providing beneficial functions for one animal (the nematode) and a pathogen, causing death of another (the insect). This combination makes it an excellent model to understand both types of relationships. To study mutualism we have identified ten genes affecting X. nematophila colonization of Steinernema carpocapsae nematodes. Six encode proteins with predicted functions in regulation or metabolism. For example, the transcription factors RpoS, RpoE, and Lrp that are required for many bacteria to respond to stress or starvation, are each required for colonization. Understanding rpoS regulation is a current goal of our research and we have identified a putative regulatory RNA, NilID RNA (nematode intestine localization), which is required for colonization and which functions, in part, to regulate the translational efficiency of rpoS. NilID RNA does not appear to be expressed under standard laboratory conditions and thus may be responding to a nematode-specific environment. Current experiments are aimed at understanding the role of NilID RNA and RpoS function in colonization and the stimuli affecting these functions. We have identified an additional three genes required for colonization, nilA, nilB, and nilC; that encode a ~10-kDa protein of unknown function, a β-barrel outer membrane protein, and an outer membrane lipoprotein. Membrane localization suggests that NilA, NilB and NilC function to link an aspect of the external environment to the inner cell. Such a function could be nutrient acquisition, adhesion, signal sensing, or some combination of these. In addition, nilA, nilB and nilC are
chromosomally linked, suggesting their products may interact. Experiments are underway to examine sub-cellular localization of each protein and their possible association with each other. Furthermore, we are assessing whether NiIA, NiIB and/or NiIC affect the expression of other genes, and how they themselves are regulated. Finally, we are searching for other factors, from either *X. nematophila* or the nema-tode, with which NiIA, NiIB, or NiIC interact.

**SYMPOSIUM (Cross-Divisional). Tuesday, 10:30–12:30.**

_You are what you eat: Multitrophism in invertebrate pathology systems_

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

**Plant mediation of bacterial disease and lethality in insects**

Gary W. Felton¹, Ibrahim Ali² and Seth Young²

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The influence of plant chemistry on bacterial disease has not received much attention in recent years. In previous studies investigators have relied upon the incorporation of specific plant chemicals into artificial diets to determine the impact of plant chemistry on bacterial infectivity. In a few examples investigators have attempted to correlate bacterial infectivity with plant chemistry. However, few if any generalities can be made from these studies and the role of phytochemistry is largely ignored as an important factor in determining bacterial activity in insects. We have utilized a two-pronged approach in determining the role of simple, plant phenolics in mediating activity of *Bacillus thuringiensis* (BT) to the boilworm *Helicoverpa zea*. First we test the effect of simple phenolics (phenylpropanoids) incorporated into artificial diet on the lethality of BT to early instars of *H. zea*. Second, we use tobacco plants with suppressed and overexpressed levels of the enzyme phenylalanine ammonia lyase (PAL). PAL expression or suppression leads to greater than 20-fold differences in the levels of phenylpropanoid phenolics in the various transgenic lines. We use this wide variation in phenolic composition to test the lethality of BT to *H. zea* feeding on the transgenic tobacco. This two-pronged approach leads to allows us to better understand the mechanism of action of phenolics in mediating BT lethality.

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

**Influence of transgenic BT plants on the performance of *Macrocentrus cingulum*, a parasitoid of *Ostrinia nubilalis***

Shannon L. Sked, Dennis D. Calvin, Consuelo De Moraes, and Nancy Ostiguy

Dept. of Entomology, The Pennsylvania State Univ., University Park, Pennsylvania 16802, USA

The non-target effects of the toxin from transgenic Bt-corn on *Macrocentrus cingulum* Brischke, a specialist parasitoid of *Ostrinia nubilalis* Hübner, are not well understood. A split plot design was used to address Bt-corn and planting date influences on parasitoid abundance. Main plots consisted of paired planting of Bt and non-Bt corn hybrids. Within each main plot, subplot patterns of corn were planted on three dates; 1 May, 15 May, and 30 May, in 2001 and 2002. *Macrocentrus cingulum* adults were captured on sticky traps placed in systematic locations within individual subplots. Traps were collected at weekly intervals throughout the growing season and the number of *M. cingulum* per trap was recorded and counted. Significantly more *M. cingulum* adults were captured in non-Bt corn plots compared to Bt corn plots; often a two to three-fold difference was observed. In addition, abundance patterns of *M. cingulum* across various non-Bt hybrid planting dates reflected abundance patterns expected of *O. nubilalis*, while abundance patterns of *M. cingulum* in Bt corn plots deviated from expected *O. nubilalis* abundance patterns across planting dates. These results suggest that Bt-corn may have a negative impact on the parasitoid *M. cingulum* in the field.

**Symposium. Tuesday, 11:10.**

**The influence of host plant on the ecology of insect-baculovirus interactions**

Jenny S. Cory

Molecular Ecology and Biocontrol Group, NERC Centre for Ecology and Hydrology, Mansfield Road, Oxford, OX7 3BW, UK

Host plant can influence insect-virus interactions in numerous ways. For example, plant architecture affects virus persistence, palatability modifies host behaviour and virus acquisition, plant chemistry modulates infection in the gut and nutrient content determines host survival. The impacts of plant phytochemicals, such as phenolics, on host susceptibility has received most attention and numerous studies have shown that virus-induced mortality varies depending on plant species. Other infection traits that could also impact on insect-virus dynamics, such as speed of kill and virus productivity, can also be influenced. However, virtually all investigations on the influence of host plant on insect-virus interaction have taken place in the laboratory. Studies which address whether host plant actually influences insect dynamics or evolution in field populations are sparse, as are experiments which estimate the effect of host plant on field-based population parameters such as transmission and virus persistence. However, most studies are host-biased; baculoviruses are intimately associated with their food plants and it is also possible that host plant could influence the virus population more directly. Recent data indicates that host plants could exert a differential effect on virus variants, which might indicate that host plant play a role in virus evolution. The influence of food plant on virus infection is complex and whether these triotrophic interactions are significant to host-virus dynamics and evolution in natural populations requires studies of transmission, adaptation and host plant usage in the field.

**Symposium. Tuesday, 11:30.**

**Plant-mediated inhibition of disease caused by baculoviruses**

Kelli Hoover, Gary Felton and Ruth Plymale

Dept. of Entomology, Penn State Univ., University Park, PA 16802

There is a growing body of literature concerning the influences of host plant chemistry on the outcome of disease caused by a variety of pathogens, including baculoviruses. For example, heliotoxins treated with *Auto grapha californica* NPV are less susceptible to mortal infection when fed cotton than lettuce, sorghum or tomato foliage. More recent studies have also examined how plants (and different plant parts such as reproductive vs. vegetative structures) affect production of progeny virus in the host insect, which is a measure of how phytochemistry influences viral fitness. In this paper, I will present what is known about mechanisms of inhibition of baculoviral disease mediated by phytochemistry. Loss of susceptibility to mortal infection by baculoviruses appears to be strongly influenced by the generation of reactive oxygen species in the insect midgut by redox cycling among plant-ingested phenolics catalyzed by plant phenolases (particularly peroxidases). Also, these chemical reactions appear to interfere not only with the transmission of the virus (primary infection), but also the spread of the infection beyond the midgut (secondary infection), primarily through increased rates of sloughing of infected midgut cells. The ability of phytochemicals to influence viral pathogenesis suggests that host plants can play an important role in the ecology of insect-virus interactions in the field.

**Symposium. Tuesday, 11:50.**

**Tri- and tetrarotrophic level effects on entomopathogenic nematodes**

Albrecht M. Koppenhöfer

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I will discuss two systems in which trophic level effects on entomo-pathogenic nematodes (EPN) have been examined. The first system is the interaction between squash, the southern corn rootworm (*Diabrotica undecimpunctata howardi*) (CRW), and EPN (*Barber chec*1993, Barbercheck et al. 1995, Barbercheck & Wang 1996). Susceptibility to *Steinernema carpocapsae* (Sc) and *Heterorhabditis*
The sugar beet root maggot, *Tetanops myopaformis* (Röder), is the most damaging insect pest of sugar beet in Minnesota and North Dakota. A study was conducted in 2002 at St. Thomas, North Dakota (Pembina Co.), for managing the sugar beet root maggot, using planting time granular and postemergence liquid applications of *Metarhizium anisum* strain MA-1200 with two cereal cover crops-oat, *Avena sativa* L., and rye, *Secale cereale* L. The integration of cereal covers with *M. anisum* is a novel approach for insect biocontrol. A split-split-plot field design was used with oat and rye cover crops as the main treatments, seeding rates (0, 1.5, and 3.0 oat bushel equivalents [OBE] per ac) as sub-treatments, with MA-1200 formulations compared to terbufos 15G and an untreated control as sub-sub-level treatments. In 2002, the relative levels of control were evaluated on a 0 to 9 damage rating (DR) scale (0 = no visible feeding injury, 9 = 75% of root surface scarred). Under the moderately to moderately severe *T. myopaformis* feeding pressure that developed (mean DR of 6.08 in untreated controls), MA-1200 provided significantly better root protection when combined with cover crops than treatments with no cover. Granular MA-1200 in the presence of oat at 3.0 OBE/ac had significantly lower root injury (mean DR=5.45) than in the absence of a cover crop (mean DR=6.70). Also, sugar beet plots receiving postemergence foliar MA-1200 had significantly less root feeding injury when combined with the rye cover at 3.0 OBE/ac (mean DR=4.5) than their non-cover counterparts (mean DR=6.22). Findings from 2002 trial indicate this novel integrated strategy suggests positive tritrophic interactions between the target insect, the entomopathogen, and the cereal cover crops that result in effective *T. myopaformis* management. The experiment will be repeated in 2003.

**Fungi – 2**

**CONTRIBUTED PAPERS.** Tuesday, 8:00-10:15.

**STU** Contributed paper. Tuesday, 8:00.

**Integration of *Metarhizium anisum* (Deuteromyotis): Hyphomycetes and Cover Crops for controlling Sugarbeet Root Maggot (Diptera: Otitidae)**

Ayanava Majumdar,1 Mark A. Boett1, Stefan T. Jaronski,2 Robert J. Dregseth,1 and Allen J. Schroeder3

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The sugar beet root maggot, *Tetanops myopaformis* (Röder), is the most damaging insect pest of sugar beet in Minnesota and North Dakota. A study was conducted in 2002 at St. Thomas, North Dakota (Pembina Co.), for managing the sugar beet root maggot, using planting time granular and postemergence liquid applications of *Metarhizium anisum* strain MA-1200 with two cereal cover crops-oat, *Avena sativa* L., and rye, *Secale cereale* L. The integration of cereal covers with *M. anisum* is a novel approach for insect biocontrol. A split-split-plot field design was used with oat and rye cover crops as the main treatments, seeding rates (0, 1.5, and 3.0 oat bushel equivalents [OBE] per ac) as sub-treatments, with MA-1200 formulations compared to terbufos 15G and an untreated control as sub-sub-level treatments. In 2002, the relative levels of control were evaluated on a 0 to 9 damage rating (DR) scale (0 = no visible feeding injury, 9 = 75% of root surface scarred). Under the moderately to moderately severe *T. myopaformis* feeding pressure that developed (mean DR of 6.08 in untreated controls), MA-1200 provided significantly better root protection when combined with cover crops than treatments with no cover. Granular MA-1200 in the presence of oat at 3.0 OBE/ac had significantly lower root injury (mean DR=5.45) than in the absence of a cover crop (mean DR=6.70). Also, sugar beet plots receiving postemergence foliar MA-1200 had significantly less root feeding injury when combined with the rye cover at 3.0 OBE/ac (mean DR=4.5) than their non-cover counterparts (mean DR=6.22). Findings from 2002 trial indicate this novel integrated strategy suggests positive tritrophic interactions between the target insect, the entomopathogen, and the cereal cover crops that result in effective *T. myopaformis* management. The experiment will be repeated in 2003.

**Ecological role of the large nettle aphid, *Microlophium carnosum*, as an early season source of *Pandora neaphidis***

P.A. Shah, S.J. Clark7 and J.K. Pell

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The study of non-crop plants as potential refugia for invertebrate natural enemies is an essential component for conservation biocontrol in agroecosystems. In northern Europe, the large nettle aphid, *Microlophium carnosum*, is an effectively monophagous herbivore whose primary resource is perennial stinging nettle, *Urtica dioica*. Nettles are common “weeds” on farmland and are larval foodplants of several high profile species of Lepidoptera.

At Rothamsted, spatio-temporal studies on the population dynamics of *M. carnosum* and aphidophagous Entomophthorales, especially *Pandora neaphidis*, have been performed using stratified leaf sampling techniques at two scales; a nettle bed (approx. 40 m²) and a cereal field perimeter (approx. 900 m²). Within the nettle bed, peak densities on tagged plants were 3, 50 and 15 living aphids leaf⁻¹ plant⁻¹ in 2000, 2001 and 2002, respectively. Peak densities of fungus infections were 0.6, 0.1 and 7.0 cadavers leaf⁻¹ plant⁻¹ during the three seasons. In the field perimeter, repeated sampling of marked nettle patches was made at 20 m intervals. Peak densities were 10 and 8 living aphids leaf⁻¹ plant⁻¹ patch⁻¹, and 4.0 and 0.3 cadavers leaf⁻¹ plant⁻¹ patch⁻¹ in 2001 and 2002. In general, host and Entomophthorales populations were synchronised, appearing in May and declining to zero by July. *Pandora neaphidis* was the most common entomophthoralean fungus in 2002 when most samples were taken. Other members of this natural enemy complex included *Entomophthora pluchoniana*, *Neozygites microlophi* and *Zooplithora phalloides* and *Conidiobolus sp.* Based on quantitative data and field
observations, *M. carnosum* numbers are influenced by aphid crowding and anthocorid predation as well as infection by Entomophthorales, while parasitism was rare at these study sites. The nittle-*M. carnosum* -Entomophthorales tritrophic interaction can be potentially useful for aphid control provided fungal infections readily disperse between non-pest and pest populations.

Contributed paper. Tuesday, 8:30.

**Tritrophic interactions between Pandora neophidis, three aphid species and different host plant resources**

P.A. Shah, S.J. Clark1 and J.K. Pell

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Studies were carried out to compare the performance of *Pandora neophidis* against aphids on standard or alternate host plant species or cultivars. The three aphid species used were the pea aphid, *Acrystosiphon pisum*, the rose-grain aphid, *Metopolophium dirhodum*, and the peach-potato aphid, *Myzus persicae*. Prior to experiments, apterous aphids had experienced short-term adaptation on alternate plants or cultivars for three to five generations.

For experiments with different host plant species, intraspecific *P. neophidis* variation was also investigated using isolates NW 343 and NW 415, classed as a “generalist” and “specialist”, respectively. Exposure of aphids to either of the two isolates was for 0.5 hr, which is a robust time to estimate as LD₅₀ in our system. The standard plants used were broad bean, barley or Chinese cabbage for *A. pisum, M. dirhodum* and *M. persicae*, respectively. The alternate plants were pea, wheat or potato. Preliminary analyses indicated that infection was significantly affected by host plant status. Predicted means were 12% (SE = 0.02) and 8% (SE = 0.01) for infection on original and alternate plant species, respectively. There was also a significant interaction between aphid species and isolate. Infection by NW 343 was almost four times higher than with NW 415 (21% c.l. 5%) against *A. pisum*. For cultivar tests, standard and alternate cultivars were used for broad bean, barley and Chinese cabbage. A period of 3 hr was used to inoculate aphids with conidia showers from isolate NW 343 to estimate as a robust LD₅₀ dose. Infections of 9-80%, 3-32% and 25-71% were obtained with *A. pisum, M. dirhodum* and *M. persicae*. In no differences in survival time between cultivars, and overall means of 3.6-6.5 days were computed. There was a quadratic relationship between survival time and dose, indicating non-linearity between *P. neophidis* performance and amount of conidia.

In summary, *P. neophidis* infection is affected by differences between, but not within, host plant species. Possible reasons for these findings will be discussed.

**Mycoinsecticide for stored product pest control**

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The maize weevil, *Sitophilus zeamais* (Motsch.) (Coleoptera: Curculi -weevils) and the larger grain borer, *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae) are the two most important and destructive storage pests in the tropics, causing both high quantitative and qualitative damage to cereals. An innovative approach was designed to develop effective and environmentally safe management strategies. To this end the efficacy of eight different mycoinsecticide formulations was assessed against *Sitophilus zeamais* and *Prostephanus truncatus*. These formulations were prepared using aerial conidia, submerged spores and submerged conidia of virulent isolates of *Beauveria bassiana* (PPRC-HH) and *Metarizium anisopliae* (PPRC-EE) obtained from Ethiopia. The efficacy test was conducted on maize grains stored at 30°C and 60-70% RH in the laboratory. The persistence of the formulations after application and their storability at 4°C and 30°C were also assessed. The results revealed that tclum and conidia-based dusty powder formulations of PPRC-HH and PPRC-EE were highly effective (efficacy = 52-100%) against both test insects. For these formulations, mortality was 40-99% at 5 days after treatment. Compared to the control, emergence of progeny was reduced by 63-96%, damage by 43-65% and weight loss by 57-85% for these treatments. Conidia: tclum: milk: molasses-based formulation of PPRC-EE also showed efficacy in the range of 44-81%. In contrast, formulations based on submerged spores/conidia of both strains showed low efficacy (< 40%). All formulations persisted for up to 5 months after application at varying levels of efficacy. Furthermore, conidia: tclum-based formulation of PPRC-HH when stored at both 4°C and 30°C maintained a high level of viability and efficacy (80-100%) for a period of 5 months. Storage of the remaining possible was at both temperatures for up to four months. However, the decline in viability and efficacy occurred at a higher rate when stored at 30°C. In general, the results obtained in this study indicate that it is possible to achieve a successful level of control for *S. zeamais* and *P. truncatus* on stored and infested maize using mycoinsecticides such as the one developed in this study.

**Use of Beauveria bassiana and its environmental effects in microbrial control of Monochamus alternatus**

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Microbial control of *Monochamus alternatus*, the most important pest insect of Japanese pine forest for transmitting the pathogen of pine wilt disease, has been studied. An isolate of *Beauveria bassiana*, F-263 was selected as the most virulent pathogen against this insect. Application of nonwoven fabric strips with *B. bassiana* conidia onto the bark of dead pine trunks to kill the larvae of *M. alternatus* under the bark was thought to be the most practical means of application methods of this fungus for control of the insect. In the field experiment with this method, 29 to 99% of the larvae could be infected with *B. bassiana*. Generally earlier applications produced higher mortality, because the larvae inhabit under the bark. However in the field, the relationship between the application timing and the mortality was not clear by the unevenness in oviposition period of this insect.

To evaluate safety of this method to other insects, conidial dispersal from a nonwoven fabric strip was investigated using a selective medium for *B. bassiana*. The conidia were dispersed by the air; however, density of the fungus in the air at more than 50 m from the source did not differ from the natural density of *B. bassiana*. Considering the lethal density of the fungus on mulberry leaves for the silkworm, the risk of infection is thought to be very rare.

To investigate impact of *B. bassiana* application on soil microorganisms, the conidia were mixed into the forest soil, and the density dynamics of soil microorganisms were investigated using selective media. The densities of bacteria and actinomyces were not affected by the addition of *B. bassiana*. Densities of both total fungi and *B. bassiana* in the treatment plot increased to 3 to 5 x 10⁵ CFU/g immediately after the mixing of *B. bassiana*. The densities gradually decreased to 1/10 after 12 months. Densities of fungi other than *B. bassiana* could not be measured in the treatment plot. However, they were not thought to be affected by mixing of *B. bassiana*, because the metabolism of *B. bassiana* seemed to be very inert in field soil, since microscopic observations revealed that *B. bassiana* conidia do not germinate in non-sterilized soil, but they do germinate in sterilized soil.

**Entomopathogenic fungi and the emerald ash borer**

Houping Liu1, Leah S. Bauer2,3, and Deborah L. Miller2

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The emerald ash borer (EAB), *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae) is an invasive pest from Asia of ash trees

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Tuesday AM
Com predetermined portable hood on first instar aestivating sistens on the new hemlock growth. Conidia of B.b. strain CA 603, isolated from avocado plantation soils did not show any effect when compared to the control groups. Conidia of V.I. strains HWA 304, isolated from HWA and SPTR 151 isolated from Eurygaster integriceps (Hemiptera: Scutelleridae) demonstrated mortality among the test insects of 36.1 ± 2.9% and 58.0 ± 3.6% respectively. In all cases, blastospores of all isolates demonstrated significantly higher effectiveness than conidia (p<0.01). These propagules were most effective when applied in combination with their cultural liquid obtained after submerged cultivation of fungi. Insect mortality was 29.4 ± 1.7% for B.b. strain CA 603, 83.1 ± 4.8% for V.I. strain HWA 304 and 100% for V.I. strain SPTR 151. The high effectiveness of blastospores in cultural liquid is likely an additive effect of their combined properties. Blastospores are a vegetative form of fungi that do not require a latent period for activation. As a result, these propagules quickly and effectively utilize the moisture associated with their carrier during application onto a plant or target species. Furthermore, cultural liquid contains a complex combination of biologically active substances that temporarily suppress the local microbial community within the habitat of the target pest, and promotes penetration of the fungus through the protective insect cuticle.
Detailed examination of the tissue at various times post inoculation did not reveal evidence of virus replication. Infected insects died 10-12 days post inoculation. An AmEPV *iap* knockout virus in which the *iap* gene was replaced with *b-galactosidase* gene under the control of the cowpoxvirus ATPI promoter was also tested for its growth and pathogenesis in gypsy moth larvae. The *iap* knockout virus, which could be propagated on Ld652 cells like the control virus, also initiates its infection in the hemolymph and thereafter spreads to the same secondary tissues but the process is delayed by 2-3 days. We conclude the gypsy moth is a suitable host in which to study AmEPV pathogenesis.

Contributed paper. Tuesday, 8:15.

**Identification of a novel baculovirus gene required for oral infectivity of insects: Pif-2**

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Oral infectivity of baculoviruses for insects is dependent on the presence of at least two viral gene products, P74 (AcMNPV ORF138 or Ac138 homologues) and a peroral infection factor, PIF-1 (Ac119 homologues). These products are associated with the envelope of occlusion-derived virion and are present in minute amounts. Infection of cultured insect cells with *Spodoptera exigua* multicapsid nucleopolyhedrovirus (SeMNPV) results in the generation of mutants with major genomic deletions. Some of these mutants lack the ability to infect *S. exigua* larvae *per os*. One mutant lacked SeORFs 15 to 35 (including genes encoding cathepsin, chitinase, gp37, ppi-2, egt, pkp-1, and arf-1). These genes appeared thus not essential for virus replication in cell culture, nor did they affect virus replication in insects as evidenced by *in vivo* intrahemocoele injection of mutant BVs. P74 (Se131) and PIF-1 (Se36) were present in this mutant suggesting that yet another gene is involved in oral infectivity. A full-length infectious clone (bacmid) of SeMNPV was generated Pijman et al. (2002). By site-specific deletion mutagenesis using ET-recombination in *E. coli*, a series of SeMNPV bacmid mutants with increasing deletions from ORF15 to 35 were generated. Analyses of these mutants indicated that a deletion of Se35 results in the loss of oral infectivity of polyhedral occlusion bodies. Reinserion of ORF35 in SeMNPV bacmids lacking Se35 rescued oral infectivity. We propose the name *pif*-2 for Se35 and its baculovirus homologues (e.g. Ac22), in analogy to a different gene recently characterized in *S. littoralis* NV, which was designated *per os* infectivity factor (*pif*). *Pif*-2 is present in all baculoviruses sequenced to date (18 genomes) and hence must play a key role in the baculovirus infection process in insects.

**Is PIF Quantity regulated by Spodoptera littoralis nucleopolyhedrovirus (SpNPV)?**

Serafin Gutiérrez1,2, Ohiane Simon1, Primitivo Caballero1 and Miguel López-Ferber1
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PIF is a baculovirus protein essential for oral infectivity of occlusion derived virions (ODV) in lepidopteran larvae. PIF has been detected only in the ODV envelope and in very low quantities. In this report, we present data suggesting that PIF low quantity is regulated by the virus. The transcription of SpNPV *pif* gene was analysed. Several transcripts overlapping *pif* nucleotide sequence were detected simultaneously at late times in infection. Among these transcripts, *pif* major transcript was shown to be a bicistronic mRNA. The relative amount of this transcript was estimated to be 300 times smaller than that of the polyhedrin gene transcript. The ORF situated immediately downstream from *pif* gene was contained in the 3′UTR of *pif* major transcript. This ORF is conserved in the sequenced NPVs. In addition, this ORF is always situated immediately downstream from *pif* gene in all the genomes, despite the fact that *pif* gene position is variable in the different genomes. A messenger resulting from the transcription of this ORF was characterised. The presence of this ORF did not seem necessary either for virus infection or for PIF function during functional complementation experiments. Most of the transcripts detected simultaneously with *pif* major transcript encompassed ORFs situated upstream from *pif* gene. The transcription of such messengers could hamper *pif* gene expression by a promoter occlusion phenomenon. This complex transcription system could modulate *pif* gene expression. Preliminary data suggest a similar transcription system for *pif* of *S. frugiperda* NPV (SpNPV). Plaque assay analysis of a wild SpNPV population revealed the presence of genotypes with deletions affecting *pif* gene, among other genes. One of these genotypes and a genotype containing the complete genome were mixed in different proportions and co-enveloped. After 4 passages of the mixtures in larva, the proportion of each genotype in the obtained offspring was similar to the proportion found in the wild population. These results suggest that a proportion of the virus genotypes within a wild population does not contain *pif* gene and that this proportion is regulated to a certain value. The results presented support the hypothesis of a specific viral regulation of PIF quantity, both at the transcription and at the population level. Further investigations will determine the reasons for this regulation.

**Site-directed mutagenesis of structural (VP) proteins of Junonia coenia densovirus (JcDNV): Impact on virus morphogenesis and infectivity**

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The genome of *JcDNV* is a linear single-stranded DNA molecule with an ambisense organization. The *vp* gene located in the 5′ half on one strand is controlled by the P9 promoter and the 4 structural polypeptides are generated from an unspliced 2.6 kb mRNA by translation initiation at the 1st, 2nd, 3rd and 4th AUG codons according to a "leaky scanning" mechanism. Protein requirements for assembly of virus like particles of *JcDNV* in insect cells was previously reported (Crozier et al. 2000). However, the role of each polypeptide in virus assembly to generate infectious particles has not been established. We report here the effect of deletions by site-directed mutagenesis of one or more polypeptides on virus infection cycle in cell culture and larvae. Six constructs were generated from pBRJ, a plasmid encompassing an infectious viral sequence, by mutating the 5′-frame ATG′s at position 555 (pJ[VP1]), 1386 (pJ[VP2]), 1521 (pJ[VP3]), 1668, and 1674 (pJ[VP4]) Plasmid pJ[VP2+3] contained the double ATG2-ATG3 mutation Each mutation generated a specific restriction site. These constructs were transfected to Ld 652 cells and infectivity tests were performed by injecting cell extracts to 3rd instar *Spodoptera littoralis* larvae. The effect of each mutation was controlled by Western blot analysis of cell extracts or purified virions and by digestion of viral DNA with appropriate restriction enzymes. Transfection of pJ[VP2] or pJ[VP3] DNA to Ld 652 cells produced virus particles with a peptide profile showing deletion of VP2 or VP3 but these virions were as infectious as wt virions when injected to *S. littoralis* larvae. In contrast, virus particles with VP1 or VP2 + VP3 deletions produced in Ld 652 cells were not infectious for *S. littoralis* larvae. No virus particles could be isolated by transfecting the pJDVP4 construct to Ld 652 cells. Finally, mutations were performed in two regions assumed to be critical: the N-terminal,VP1-specific sequence containing a phospholipase A2 activity and a Lysine-Arginine-reach region close to the N-terminal sequence of VP2. Both mutations drastically reduced infectivity of mutant virions. Taken together these results demonstrate the non essential role of VP2 or VP1 in virus assembly and infectivity and confirm the essential role played by VP4 in virus morphogenesis and by VP1 in virus infectivity. Ref.: Crozier L., Jousset F.X., Veyrunes J.C., Lopez Ferber M., Bargoin M., Croziez G. (2000). *J. Gen.Viral*, 81, 1605-1613.
Analysis of a zinc-finger protein from *Choristoneura fumiferanae* nucleopolyhedrovirus

José de Jong 1, Bassi Arif 2 and Peter Kret 1

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The *Choristoneura fumiferanae* multicapsid nucleopolyhedrovirus (C-MNPV) is an ideal candidate as a bioinsecticide to control the eastern spruce budworm (*C. fumiferanae*) due to its narrow host-range. We have identified a C-MNPV unique ORF (CfU4) that encodes a putative DNA binding domain. CfU4 has an estimated molecular mass of 14.02 kDa and contains a putative zinc-finger domain. Temporal transcriptional analysis has indicated that CfU4 is transcribed within the first six hours of viral infection. Northern blot analysis results demonstrated two major transcripts peaking at approximately 72 hours post-infection. We have identified a single transcriptional start site and a transcriptional termination site using 5’ and 3’ RACE. We have also generated a CfU4 knockout mutant through homologous recombination with a GFP marker indicating that CfU4 is not essential for viral replication in C-203 cells. Viral growth curves and temporal expression analyses have indicated that the null-mutant behaved similarly to wild-type C-MNPV. We are currently using real-time PCR to quantify any difference seen in transcriptional levels of the immediate early, early, late and very late transcription classes and to quantify differences in the kinetics of viral DNA replication.

Contributed paper. Tuesday, 9:15.

A bacmid of HaSNPV with a 20-kb deletion is still infectious

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During the construction of Bac-to-Bac system of *Helicoverpa armigera* single nucleocapsid nucleopolyhedrovirus (HaSNPV), many bacmids with large deletions in the genome were obtained. Here we report the study of one of the bacmids, named HaBacH211, which carries about a 20-kb deletion in the genome. In order to study the infectivity of the bacmid, the polyhedra gene and eGFP gene were transposed to the Tn7 attachment site on the HaBacH211 and generated recombinant HaBacH211PHeGFP. The DNA of the recombinant was transfected into HaZAM1 cells, coculosis bodies and green fluorescent were found in the HaZAM1 cells in 5-7 days after transfection. One-step growth curve of the recombinant virus indicated that HaBacH211 was infectious to HaZAM1 cells. The electron micrographs analysis revealed large amount of virions were produced in the nucleuses of infected cells, but they were not properly occluded into polyhedra. Bioassay was conducted with polyhedra of HaBacH11PHeGFP in comparison with that of HaBacH8PHeGFP and wt-HaSNPV by oral infecting *H. armigera* larvae. Although HaBacH11PHeGFP had a higher LD50 and a lower ST50 than the control viruses, it did infect different tissues of *H. armigera* larva and could kill the host at the end. All the above data indicated that the in spite of about 20-kb deletion in the genome, the bacmid is still infectious both in vitro and in vivo. A detailed mapping of the 20-kb deletion is carrying out in our laboratory.

Contributed paper. Tuesday, 9:30.

Trypsinization of occlusion body-derived virus from three nucleopolyhedroviruses alters infectivity to insect cells lines

Dwight E. Lynn

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Twelve insect cell lines were tested for susceptibility to baculovirus infection by use of a typical endpoint assay procedure using alkali-labeled occlusion body-derived virus (ODV). Cell lines from *Spodoptera frugiperda* (IPLB-Sf21AE), *Anticarsia gemmatalis* (UFL-Ag286), *Heliothis virescens* (IPLB-HvE1a, IPLB-HvE6a, IPLB-HvE6b and IPLB-HvT1), *Lymnaea dispar* (IPLB-LdE1a and IPLB-LdE1b), *Plutella xylostella* (IPLB-PxP21), and *Trichoplusia ni* (COS-3681, IAL-TN1D, and IAL-TN-R1) in 96-well tissue culture plates were each infected with dilutions of ODV from three nucleopolyhedroviruses (NPVs), including *Autographa californica* NPV (AcMNPV), *Anaglyphra falcifera* NPV (AFMNPV), and *A. gemmatalis* NPV (AgMNPV). Additionally, samples of the ODV of each virus were treated with trypsin (0.05 mg/ml) prior to inoculation of cells (ODV-T). The result of virus titers reveal the relative infectivity of ODV and ODV-T from the three viruses to each cell line. Trypsin causes a slight (2- to 5-fold), but consistent, increase in infectivity for each virus in many of the lines tested. Of particular interest, however, are the results with AcMNPV and AFMNPV on the LdEp and TN-368 lines. Contrary to results on most lines, the titers in LdEp are always slightly lower with trypsin than without, although this line also has the highest level of susceptibility to ODV from AcMNPV and AFMNPV and is second only to Ag286 with AgMNPV ODV-T. Alternatively, AcMNPV and AFMNPV ODV show a very large (over 5,000-fold) increase in infectivity to the TN-368 line after trypsinization. Since proteases are a natural feature in the insect midgut where ODV typically initiates the infection of the insect, these results with trypsin might be expected. However, the mode-of-action is currently unclear and will be the focus of further investigation.

Contributed paper. Tuesday, 9:00.

TUESDAY AM

SYMPOSIUM (Div. of Fungi), Tuesday, 10:30–12:30.

Challenges to the use of fungi for control of Acari

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The twospotted spider mite, *Tetranychus urticae*, is an important pest of crops world-wide. In response to pesticide resistance, farmers and growers have increased their use of biological control, which is done by conserving natural enemies and/or by applying predatory phytoseid mites. However, this is often not effective on its own, and supplementary acaricide sprays are used routinely. On crops such as tomato, differences was the establishment and developmental rates between the predator and the prey have resulted in a strong dependency on a small number of acaricides for spider mite control. Consumer fears about pesticide residues and concerns about the development of resistance to the remaining pesticides has meant that the development of an alternative to chemical sprays has become increasingly important.

We evaluated 40 isolates of entomopathogenic fungi against *T. urticae* feeding on tomato in laboratory bioassays. Only three isolates caused significantly more mortality than the controls. Further investigations, using a subset of isolates, indicated that the virulence of *Beauveria bassiana* was significantly affected by the inoculation method used in the bioassay. In a glasshouse experiment, sprays of *B. bassiana*, *Hirsutella thompsonii*, *Metarhizium anisopliae*, *Verticillium lecanii*, and the *B. bassiana*-based product Naturalis-L (Troy Biosciences USA) significantly reduced *T. urticae* populations on a tomato crop grown according to commercial practice. A second glasshouse experiment was done to compare Naturalis-L against the chemical acaricide fenbutatin oxide, as supplementary sprays to the predator *Phytoseiulus persimilis*. Fenbutatin oxide significantly reduced numbers of *T. urticae* nymphs, while Naturalis-L significantly reduced numbers of *T. urticae* eggs, nymphs and adults. Both treatments were compatible with *P. persimilis*. Further research is required to develop a more effective bioassay system and identify a wider range virulent fungal isolates.
Challenges in using *Neozygites tanajae* as a classical biological control agent for the cassava green mite in Africa

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F. C. C. Hountondji\(^4\) and A. Cherry\(^5\)

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In the past 14 years, Brazilian isolates of the fungus *Neozygites tanajae* (Zygomycetes: Entomophorales) have been investigated as a control agent for the cassava green mite (CGM), *Mononychellus tanajae*, in Africa. There have been few attempts to use entomopathogenic fungi for classical biological control, and the majority of cases consisted of introduction of species to areas where they did not already occur. The fact that ineffective endemic strains of *N. tanajae* occur in parts of Africa makes the implementation of a control program challenging. Moreover, *N. tanajae* is particularly difficult to isolate and to produce in *vitro*, and this has limited other important studies, e.g., selection of isolates and molecular characterization for pathogen detection. Culture media for production of hyphal bodies of this fungus were not developed until 1999, and sporulation from *in vitro* cultures is restricted. For these reasons, only a limited number of attributes could feasibly be used for selection of isolates using capilliconidia produced from mummified CGM. Acquiring necessary permits for pathogen importation and release has been increasingly difficult in many countries. This was not an issue with *N. tanajae* in Africa because this pathogen presents desired attributes of a classical biological control candidate. *N. tanajae* is specific to CGM, endemic strains of this species already exist in Africa and exotic strains appear to have no impact on non-target organisms. *N. tanajae* seems to survive as resting spores when the host is absent and during dry seasons, suggesting that resting spore formation is critical for successful establishment of the pathogen in new areas. However, the factors inducing resting spore formation and germination are not well known, so releases have to be conducted using live infected mites from small scale laboratory production. Isolates from Brazil were released in Benin (*Africa*) in 1998 and 1999 but molecular probes to distinguish exotic from native strains were still not available, making it difficult to confirm its establishment. Sequencing, random amplified polymorphic DNA (RAPD) and amplified fragment length polymorphism (AFLP) revealed low polymorphism among Brazilian and African isolates. We have determined a few RAPD polymorphic markers that can potentially be used to detect the exotic isolates. Based on these markers molecular tests are currently been optimized to determine the outcome of the releases in Africa.

Novel strategies for control of chicken mites

(Dermanyssus gallinae) using autodissemination

Tove Steenberg

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The chicken mite, *Dermanyssus gallinae*, is the number one pest in commercial egg production in Europe and can be found world-wide. This bloodsucking mite feeds on the hens at night and aggregates during the day in cracks and crevices in the poultry house. Severe mite infestation results in reduced welfare and productivity in the flocks, and often causes considerable skin irritation in farm workers. In several European countries none or only a few pesticides are registered for chicken mite control, there have been reports of pesticide resistance, and the increasing number of organic farmers have no efficient control methods. In a collaborative European project partners from Spain, United Kingdom and Denmark attempt to develop a new control method based on the lure-and-attack approach, where the ultimate aim is to combine an attractant semiochemical and an entomopathogenic fungus in an autodissemination device. There are no records of naturally occurring entomopathogens in *D. gallinae*; however, the mites are susceptible to infection by entomopathogenic fungi. Bioassays, where suspensions of 2 x 10^{3} conidia ml^{-1} of 13 fungal isolates (*B. bassiana*, *M. anisopliae*, *V. lecanii* and *P. fumosoroseus*) from different sources were tested against engorged females, showed that 11 isolates caused mortality in *D. gallinae*. At this concentration even the most virulent isolates only caused mortalities of 54%-75% within 12 days of incubation at 25°C. In maximum exposure tests with dry conidia the best isolate caused >95% mortality within 6 days. Research is underway to study the transmission potential of the most virulent isolates to evaluate the possibilities for autodissemination. Ongoing work in the initial part of this project also includes studies of the fecundity of infected mites, and of the persistence of conidia at high levels of ammonia.

Fungi for control of ticks

Michael Samish*, Galina Gindin* and Iamar Glazer*

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There are about 850 tick species which ingest only blood and stay roughly 20% of their life cycle on the ground. Ticks inhibit a very large variety of ecological niches. Fungi were reported to be the major pathogen of ticks in nature.

All tick stages, including their eggs, were generally found to be susceptible to fungi mainly from the genera *Beauveria* and *Metarhizium*. The fully engorged female ticks were often found to be more susceptible in comparison to the other engorged or unengaged stages. In most cases, the smaller the tick stage the shorter it’s lethal time. Even though all tick species tested were found to be susceptible to entomopathogenic fungi, the differences in degree of susceptibility is very large. Similarly the virulence of different fungi species and strains to ticks also differ markedly.

The very few experiments published on spraying conidia on either tick-infested field areas or on tick-infested vertebrate hosts demonstrated, in most cases a significant reduction in the tick population. However, the reduction and/or the time span of conidia activity was not sufficient. Finding an optimal fungus strain and developing a satisfactory formulation seems to be the main key for obtaining a successful commercial compound.

Evaluation of entomopathogenic fungi for control of *Varroa destructor*, an ectoparasite of the honey bee, *Apis mellifera* L.

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The varroa mite, *Varroa destructor* is a damaging ectoparasite of the European honey bee, *Apis mellifera*. It originates in Asia, but has extended its range and is now causing severe damage to *A. mellifera* populations throughout the world. Adult female *V. destructor* feed on the haemolymph of honey bee pupae and adults and can activate and transmit honey bee viruses, causing a decline in pollination efficiency and honey production. At present, beekeepers attempt to control varroa with chemical pesticides, but resistance is developing and alternative methods of control are required urgently.

We are investigating entomopathogenic fungi as potential microbial control agents of *V. destructor*. A laboratory bioassay was developed to measure the susceptibility of adult mites, feeding on bee pupae, to suspensions of fungal conidia. Forty isolates of fungi from six genera were evaluated in a single-dose experiment (1 x 10^{6} ml^{-1}) at 25°C / 100% R. H. All the isolates killed *V. destructor* and 26 caused mean times to death of less than 100 h. Nine isolates were evaluated further in bioassays at 30°C / 40% RH (1 x 10^{7} ml^{-1}) to simulate the conditions in honey bee colonies. Five of the isolates caused 100% mortality within 7 d. An isolate of *M. anisopliae* killed 97% of *V. destructor* within 7 d at a concentration of 1 x 10^{7} ml^{-1}. Because high temperatures are likely to be a major abiotic constraint on fungal activity in honey bee colonies, isolates showing desirable responses to high temperature were selected using a nonlinear model of poikilotherm development. We have also measured the effect of
candidate isolates on non-targets, including honey bees, ladybirds and predatory mites. The response against the non-targets varied with isolate, and candidates with favourable responses were selected. Further work has been started to assess a subset of isolates against G. destructor feeding on caged populations of A. mellifera. Research is also underway to evaluate isolates for environmental risk assessment, mass production, and response to the environment of the honey bee colony.

**POSTERS – 2**

**VIRUSES**

**Poster / Viruses. V-1.**

A new densovirus isolated from the african cotton bollworm, *Helicoverpa armigera* Hbn. (Lepidoptera: Noctuidae) in Egypt

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Recent studies revealed that the members of the Densovirus genus (DNV) belonging to the specific subfamily of invertebrate Densovirinae of the Parvoviridae family showed remarkable high virulence and wide host range for possible use as a viral biopesticide against insect pests. For studying the genomic diversity of the Egyptian densovirus isolate from *Mythimna loreyi* (MDNV), the epidemiological survey was done on the noctuid fauna of the lucerne alfalfa *Medicago sativa* on which the pests are the same that attack the cotton fields. During this work, we have isolated from dead larvae of the African Cotton Bollworm *Helicoverpa armigera* another 25nmicosahedral non-enveloped DNA virus sharing the main biological and biophysical properties of densovirus that we named HaDNV. After characterization and partial cloning of the genome, the presence of antigenic cross-reactivity and some sequence homology indicates that HaDNV and MDNV are not phylogenetically distant. The 6 kb genome of HaDNV was found to have high homology with members of the Densovirus genus as *Galleria mellonella* DNV, Junonia coenia DNV and MDNV.

**Poster / Viruses. V-2.**

Allotropic determinants of *Galleria mellonella* and *Mythimna loreyi* densoviruses reside on the viral capsid protein

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In contrast to most known viruses, autonomously replicating parvo-virus tropism seems to be controlled by intracellular factors rather than by cell receptors. Allotropic determinants thus far are localised in the VP coding sequence suggesting a critical role for the VP during infection. In the present work, we are looking to locate the tropism determinants for two closely related insect paroviruses, *Galleria mellonella* and *Mythimna loreyi* densoviruses, *GmDNV* and *MfDNV*, respectively. The two viruses share more than 90% of sequence identity and are identical for the overall genome organization. However, they differ dramatically in their host range in vivo, *GmDNV* is restricted to its host *Galleria mellonella* whereas *MfDNV* is polispecific infecting several insect species within order Lepidoptera. Using two infectious clones, pGm4 derived from *GmDNV* (Tijsen et al., in press) and pMf28 derived from *MfDNV* (Fédère, non-published data), we found a similar phenomenon for their tropism in vitro. On three different cell lines, LD-652, SL-52 and SF-9, the *MfDNV* was polispecific, infecting the three cell types, whereas *GmDNV* was only infectious to LD-652 cells. We used the two infectious clones to create several chimeric constructs between the two viruses by swapping domains within the NS and VP coding sequences as well as the terminal repeats that contain viral promoters. Using confocal microscopy and flow cytometry, our results on the phenotypic effect of these chimeras showed that the homologous exchange of viral promoters as well as NS coding sequences between the two viruses were not sufficient to confer the viral tropism of *GmDNV* clones to SF-9 cells. Whereas, exchanging the entire *GmDNV*-VP sequence by its homologue of *MfDNV*, and vice-versa, conferred the phenotype of each virus to the other. The reversion of pathogenicity was found to be located in the N-terminal of the VP, between residues aa1 and aa 193. That region of the *MfDNV*-VP, which contains 27 different amino acids from that of *GmDNV*, is located in the VP up, which was previously reported to carry an enzyme domain (phospholipase A2). Site-directed mutagenesis on that N-terminal residue is currently in progress in order to determine the minimal amino acids that govern the viral tropism.

**Poster / Viruses. V-3.**

Gene organization and content of the Neodiprion lecontei NPV genome

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The gene content and organization of the genome of the nucleopolyhedrovirus from the redheaded pine sawfly, *Neodiprion lecontei* (NeleNPV), were investigated and compared to other baculovirus genomes so far sequenced. NeleNPV is one of two hymenopteran baculoviruses presently being investigated. It is the smallest baculovirus genome so far sequenced, containing only 81,756 base pairs. Due to very low amino acid identity, homologues to other baculovirus ORFs were difficult to identify. Ninety potential ORFs were accepted with only 47 being clearly identified as genes. Of these 41 showed baculovirus matches, one had similarity to a densovirus protein and five were identified based on the presence of conserved domains. Several ORFs showing a baculoviral match were closer to ORFs from GV's than to those from NPV's. The conserved core of baculovirus genes has dropped to 29 as NeleNPV appears to lack an Ld130 homologue or F-protein. This raises the question of whether an extracellular virus phenotype is part of this virus makeup. Typical homologous repeat regions were not found but seven direct repeat regions were identified. Gene parity plots showed the only region conserved in NeleNPV compared to all other fully sequenced baculoviruses, was the helicase Ief-5 cluster. The overall average amino acid identity of clearly identified NeleNPV ORFs with other baculoviral genomes ranged from 19 to 23%. Polyhedrin, normally a highly conserved protein, showed only 45% amino acid identity with the AcNPV polyhedrin. A phylogenetic tree of all baculovirus conserved ORFs shows NeleNPV in an out group as is CuniNPV, suggesting that the hymenopteran and dipteran baculoviruses existed before the lepidopteran NPV and GV split.

**Poster / Viruses. V-4.**

Structural- functional analysis of the Apoptosis Suppressor Protein P49 from the Spodoptera littoralis nucleopolyhedrovirus

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The *Spodoptera littoralis* nucleopolyhedrovirus (SINPV), encodes a 49 kDa apoptosis suppressor protein which displays 48.8% identity to P35 of AcMNPV. Computer-assisted modeling of P49 based on the structure of P35 predicted seven [3]-helical motifs, three of them unique to P49. The structure includes a reactive site loop (RLS) protruding from a [3]-barrel domain that begins at the [3] helix. To identify domains important for P49’s anti apoptotic function we performed site directed mutagenesis and studied the effect of those mutations on the ability of P49 to suppress apoptosis in SF9 cells and to bind and inhibit insect caspases. Our results suggest that P49 and P35 bear a
scaffold common to baculovirus suppressors of apoptosis, that the [-helical regions [1, 2 and 3 are required for P49's anti apoptotic function and, that some of the unique motifs in P49 are imported for caspase - recognition.

**Poster / Viruses. V-5.**

**H2-2V genome analysis**

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H2-2V is a viral pathogen, which causes the sterility in Helicoverpa zea moths. H2-2V is a non-occluded, enveloped, rod-shaped virus, which contains double stranded circular genomic DNA. The 231,621 bp viral genome has 115 putative open reading frames, and closely resembles H1-1 virus in DNA sequence homology and genomic organization. The G+C content of H2-2V is 41.9% with a coding density of one gene per 2kb. Sequence analysis using the GenBank database identified 29 open reading frames (ORFs), of which 18 ORFs showed significant homology to proteins of known function. Some of these identified ORFs are related to known genes involved in DNA replication, (DNA polymerase and ligase), RNA transcription (VLF-1, LEF-8), and apoptosis inhibition. Some baculovirus structural protein gene homologs were identified (pvd-e56, p74, and p91) while the H2-2V structural protein genes (p11.7, p31.7) identified by mass-spectroscopy did not show significant homology to any known gene sequences. Interestingly, many homologs to cellular genes were also identified in the H2-2V genome including carboxylesterase, ribonucleotidereductase, serine, deoxyribonucleosidereductase, dihydrofolatereductase, and zinc metalloproteinase from Drosophila, Aedes and Heliotris. H2-2V also contains several additional baculovirus gene homologues (AcORF-22, AcORF-98, AcORF-119, PxORF-109, and PxORF-29), however, the relationship between H2-2V and baculoviruses based on gene content and organization, is still unclear.

**Poster / Viruses. V-6.**

**Alteration of the development of reproductive tissues in H2-2V infected Helicoverpa zea**

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Replication of H2-2V occurs in the reproductive tissues of Helicoverpa zea. Virus replication results in the malformation of these tissues and in females this is accompanied by the hypertrophy of the common and lateral ovarids and proliferation of the cells that comprise these tissues. To determine when during development, this malformation occurs we have examined tissues destined to become reproductive tissues in last instars and early pupae. In normal females these tissues fuse forming a branched structure which shrinks and then differentiates into the oviducts and the other reproductive tissues. In infected females the fusion of these tissues is delayed and results in tissues that are more dense and compact and remain large. Although there is very little evidence for virus replication in insects during this time in development we have detected differences in esterase activity in these tissues and differences in protein profiles using 2D gel analysis.

**Poster / Viruses. V-7.**

**Altered mating behavior and pheromone production in female Helicoverpa zea moths infected with the insect virus H2-2V**

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H2-2V replication in the reproductive tissues of female Helicoverpa zea results in the malformation of these tissues and the accumulation of virus forming a "waxy" plug over the reproductive opening of infected insects. We have found that infected females with this virus plug exhibit calling behaviour and attract more males in flight tunnel experiments than normal females. One reason for this increase in attractiveness is that pheromone glands from virus infected females produce 6 to 7 times more pheromone than glands form normal females. The structure and the composition of the pheromone glands and the cells that make up these glands are currently being examined to determine why viruses infected females produce more pheromone than controls.
the efficacy of HearNPV on cotton when tested in a similar way but the virus was not effective when larvae were allowed to feed on cotton leaves treated with the virus indicating that internal factors were responsible for the effect. Hear was unaffected by exposure to the leaves of tomato. Studies are currently underway to determine the component(s) responsible for HearNPV inactivation using selective bioassay and analytical chemistry. The qualification of the HearNPV inactivating factors will help to determine improved formulations with enhanced field persistence of the virus. This will reduce virus production costs, maintain yields and help manage increasingly insecticide resistant pod-borer larvae on farms in developing countries such as India and Nepal as well as developed countries like Australia. The outcome of this research will emphasise the use of innovative alternatives such as the non-chemical management of crops and help pest management become more environmentally conscientious.

Poster / Viruses. V-10.

Defective baculoviruses increase the pathogenicity of the virus population
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Natural populations of nucleopolyhedroviruses comprise mixtures of genotypes. Analysis of the insecticidal activity of NPV populations usually show lower pathogenicity than some of the pure genotypes present in the population. This may be due to the presence of deletion mutants behaving as parasites in the population. Analysis using plaque assay of a natural population of a nucleopolyhedrovirus infecting Spodoptera frugiperda (SFMNPV) revealed the presence at least of nine genotypes, distinguished by their restriction profile pattern. Genotype B was the most similar to the wild type population. Genotypes C and D were deletion mutants. The biological activity of each genotype was analysed. The potency of B genotype was only 37% of that of the wild type. Defective genotypes C and D were not infectious per os, but retained infectivity by injection into the larval haemocoel. These defective genotypes are unpaired in the pif gene, previously shown to be responsible for occlusion body (OB) per os infectivity. Mixtures of OBs from B and C variants in various proportions did not permit recovery of the C variant in the dead larva; viral DNA from all dead larvae showed exclusively a pure genotype B restriction pattern. The L50 of OB mixtures corresponded exactly to the proportion of the B genotype present. Experimental virus populations were constructed by injecting mixtures of virions of genotypes B and C in various proportions into host larvae. In bioassays, the polyhedra of mixed B+C populations revealed that the presence of genotype C in proportions of approximately 25% restored the insecticidal activity to wild-type values. From those results it is clear that B genotypes act as helpers for the C genotypes facilitating virus entry into the larvae, but C genotypes are important for the pathogenicity of the virus population. B and C genotype proportions were analysed during four successive passages of experimental populations. The proportion of deletion genotypes evolves towards an equilibrium corresponding to their proportion in the natural population. In certain situations, defective genotypes appear to play a positive role in virus populations. These findings must be taken into account when developing baculovirus based bio-pesticides. Pure genotypes are likely to be far less effective than genotypic mixtures.

Poster / Viruses. V-11.

Localization and sequence analysis of the Anticarsia gemmatalis nucleopolyhedrovirus 25K FP gene
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The in vitro production of several baculoviruses is still a strong requirement on a commercial perspective of their use as insecticides. However the accumulation of genotypic variations by serial passage in cell culture is a strong limitation. One of the most interesting aspects of the viral passage is the change from the parental, many polyhedra per cell (MP) phenotype to the few polyhedra per cell (FP) phenotype. The major problem of the passage effect is the reduced occlusion production and loss of virulence of the occluded virus (PIB). Frequent mutations have been identified within a specific region in the FP mutants that contains the 25K FP locus. This gene encodes a 25KDa protein that is essential for virion occlusion and polyhedra formation. In Brazil the Anticarsia gemmatalis nucleopolyhedrovirus (AgMNPV) has been extensively applied on soybean crops to control the velvetbean caterpillar. Production of the virus is currently done by in vivo infection of caterpillars on the field. In this work the 25 K FP gene of the AgMNPV 2D, a plaque purified virus, was identified and sequenced. Localization was done by Southern hybridization in which electrophoretically separated AgMNPV DNA restriction fragments was probed with the 25K FP gene of Helicopera armigera SNPV. Signals of hybridization were produced for HindIII-R/HindIII-S (comigrated in the gel), EcoRI-B, BstEl-B and PstI-A fragments of AgMNPV. The complete nucleotide sequence was performed by dye terminator chemistry method after subcloning fragments of a HindIII library. The 25K FP gene opening reading frame of AgMNPV is 627 bp, encoding for 208 amino acids. It was found that 94% of the gene is aligned in the HindIII-S fragment (N-terminal) and 530 bp in the HindIII-R fragment (C-terminal). The following identities were obtained from our sequence alignment of 25K FP protein from other nucleopolyhedroviruses: 87% with Epiphysys postvitiana MNPV, 83% with Orgyia pseudotsugata MNPV, 75% with Autographa california MNPV, Rachiplusia ou MNPV and Bombyx mori MNPV and 60% with Helicopera armigera SNPV. The characterization of the 25K FP gene of the wild type AgMNPV is part of our current studies on the passage effects of this virus in two different cell lines.

Poster / Viruses. V-12.

Baculovirus susceptibility, improved protein production, and resistance to nutrient stress by new Trichoplusia ni (BTI TnSB1-4) High Five™ cell clones
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Two clonal cell lines, highly susceptible to Autographa californica MNPV, were obtained from the parental cell line, High Five™ at passage 90. Both clones, designated as HSCI-B and HSCI-F, exhibited a distinct morphology and a similar growth rate in serum-containing TNMFL medium. Clone HSCI-B was remarkably resistant to nutrient stress in phosphate buffered saline while both clones were highly resistant to Actinomycin D. Both HSCI-B and HSCI-F clones produced 30 and 45% more recombinant beta-galactosidase than the parental High 5 cells. Similarly, both clones produced 100% more secreted alkaline phosphatase than the parental cells. Since High 5 cells have been considered as the highest producer among currently available lepidopteran cell lines, these distinct characteristics with the HSCI-B and HSCI-F clones could provide significant application in large-scale production of recombinant proteins and wild-type viruses.


The effect of baculovirus infection on the translational machinery of lepidopteran host cells
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The synthesis of host proteins is shut off progressively during baculovirus infection, whereas late and very late viral genes are still highly expressed. Many viruses including picorna-, adeno-, and herpesviruses control translation by modulating host cell initiation
Factors. Research was directed towards the identification of translation initiation (eIFs) and elongation factors (eIFs) in lepidopteran insect cells, and the effect of baculovirus infection on these proteins. cDNA sequences were obtained for the cap-binding protein eIF4E, the hypusine-containing protein eIF5A, the alpha sub-unit of eIF2 - which is part of the AUG recognizing complex - elongation factor eIF2, and several ribosomal proteins. The mRNA level for all these proteins was strongly reduced in Sf21 cells at 24 h post infection with AcMNPV, while reduced eIF4E and eIF2-alpha protein levels were found at 48 h p.i. Translational control via modulation of the phosphorylation status of eIF4E was not observed during baculovirus infection. Transcripts for ribosomal protein L15 and eIF2 were shown to contain a 5' terminal oligopyrimidine (TOP) tract. According to our data, using eIF2 and ribosomal protein L15 as a model, TOP mRNAs are controlled in a similar way as other host mRNAs during baculovirus infection (in contrast to the situation in herpes virus infections). All host mRNAs tested were translated well into the late stage of baculovirus infection (16 h p.i.). The coordinate disappearance of the various host mRNAs, via a yet unknown mechanism, appears to be the main regulating factor in baculovirus-induced host shut off.


Reflex bleeding, a transmission mechanism induced by baculovirus infection in the butterfly Heliconius hutora (Nymphalidae: Heliconiinae)

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A novel baculovirus (HhMNPV) has been isolated from the neotropical butterfly Heliconius hutora. Electron microscopy of HhMNPV-infected tissues indicated that nucleocapsids of this virus are multiply enveloped and are embedded in relatively small occlusion bodies. Although H. hutora larvae are highly susceptible to infection by HhMNPV, they do not display the typical terminal walking symptoms associated with many baculoviruses. HhMNPV infection stimulates a reflex bleeding response not observed in healthy larvae. Reflex bleeding, resulting in the release of infectious occlusion bodies, may enhance horizontal transmission of HhMNPV.


Purification and characterization of two viral particles from diseased postlarvae of Macrobrachium rosenbergii

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A disease of Macrobrachium rosenbergii, the giant freshwater prawn farmed in China was recently recorded in the Zhejiang, Jiangsu, Shanghai, Guangxi and Guangdong provinces. Clinical signs of the disease, which develops in postlarvae (PLs), correspond to a whitish appearance of the muscles, particularly noticeable in tail (abdomen). Mortalities may reach 100% in some hatcheries. Investi-gations by negative TEM staining on diseased PLs homogenates showed the presence of 2 types of particles: the first one, un-enveloped, icosahedral in shape, 26-27 nm in diameter, the second, much smaller, about 14-16 nm in diameter, called extra-small virus particle (XSV). By analysis of the total cell RNA extract in agarose gel electrophoresis, 5 bands (3.0, 1.2, 0.9, 0.9 and 0.85 kb) were observed. Purification and separation of two viral particles were success-fully performed by sucrose and CsCl gradient density centrifugation. The larger particle had a mean buoyant density of 1.32 g/cm3 in CsCl and contained two ssRNA segments with the size of 3.0kb and 1.2kb respectively. By its characteristics it is strongly related to the Nodaviridae family. And the smaller one had a mean buoyant density of 1.33 g/cm3 in CsCl and contained single ssRNA segment. By its very small size and its hypothesized biochemical and biological characteristics, this virus appears as a new type of crustacean virus.

Poster / Viruses. V-16.

A possible transmission pathway in vivo of white spot syndrome virus

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White Spot Syndrome Virus (WSSV) is a fatal pathogen to most of aquatic crustaceans. Little is known about its transmission in vivo and immune reaction of its hosts. Crayfish Procambarus clarkii is one of alternative hosts of WSSV. In this study, viral propagation ability in circular haemocytes of crayfish Procambarus clarkii have been investigated. Circular haemocytes of WSSV infected animals and WSSV inoculated haemocytes were collected, fixed and sectioned, then observed by using TEM histological method and in situ hybridization. In ultra-sections of infected haemocytes, enveloped virions were phagocytosed in cytoplasm, and no viral particles were observed in infected haemocytes nuclei. The results of hybridization in situ with WSSV probes also demonstrated that there are no positive signals present in haemocytes. Viral inoculated haemocytes in different post-infection times were subsequently injected to healthy crayfish and caused high mortality. WSSV particles were then observed in these haemocytes injected animals hemolymph. Our results indicated that WSSV couldn’t propagate in circular haemocytes of Procambarus clarkii. Phagocytosed virions in haemocytes may evade from host haemocytes and transfer to their target tissues with circular hemolymph. This indicated a possible transmission pathway in vivo of WSSV.

Poster / Viruses. V-17.

A novel envelope protein which is involved in white spot syndrome virus infection

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White Spot Syndrome Virus (WSSV) is a major disease agent of shrimps since it was found in south Asia in 1990s. It is at present a fatal pathogen to shrimp industry worldwide. Moreover, its wide host range makes it to be a potential pathogen to other crustaceans such as crab and crayfish. To date, most of known genes focused on viral structural protein genes and conserved enzyme genes based on proteomic methods and sequence alignment. The gene function studies were greatly retarded because of lacking any WSSV permissive cell line. Only a few gene’s function were investigated in vivo or by using baculovirus expression system. In this paper, we analyzed one ORF (designated as vp76) of WSSV which contains one conserved motif of eukaryotic cytokine receptor gp130. This ORF contains 2025 nucleotide and codes for 675 amino acid with a theoretical molecular weight of 76 kD. The gene product was expressed and purified in E. coli, then used as antigen to produce antibody. This protein was then identified to be a novel envelope protein by SDS-PAGE and western blot. It’s function was further investigated by neutralization experiment with specific vp76 antibody. The result showed that antibody neutralized WSSV lost its virulence to its host. Thus, vp76 is a protein involved in WSSV infection.


Absence of PIF blocks baculovirus ODVs infection after the binding step

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Baculovirus ODVs enter into the brush border midgut cells by direct fusion of the virus envelopes to the cell membranes in a two step mechanism (Horton and Burand 1993). Two genes have been described that abolish the per os infectivity of ODVs, p74 (Kuzio et
Invasion process of Culex nigripalpus nucleopolyhedrovirus (CuniNPV) in midguts of larval mosquitoes

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Development of Culex nigripalpus nucleopolyhedrovirus (CuniNPV) is restricted to the nuclei of midgut epithelial cells in mosquitoes. Similar to other baculoviruses, it has two virion phenotypes, an occluded form (ODV) that initiates infection in midgut epithelium and a budded form (BV) that spreads the infection within the midgut. In the presence of Mg\(^{2+}\), CuniNPV is readily transmitted via ODV to all 4 instars of mosquito larvae and normally results in death 72-96 hours post-infection. The role that Mg\(^{2+}\) plays in the infection process is unknown. Therefore, we have conducted time course studies to determine when Mg\(^{2+}\) is required for successful invasion of CuniNPV in mosquito midguts and where it may be functioning.

Larvae of Culex quinquefasciatus were exposed to CuniNPV with 10 mM Mg\(^{2+}\) for 2, 4, 6, 12, and 24 hr periods. Percent infection were determined for each group 48 hours post-exposure. Maximum infection levels were reached with 4 hours of exposure indicating that the requirement for Mg\(^{2+}\) was restricted to the early events in the infection process. Time-series ultrastructural studies with and without Mg\(^{2+}\) for the first 2 hours of the infection process determined that Mg\(^{2+}\) was not required for release of ODV’s from CuniNPV occlusion bodies in the midgut lumen. Mg\(^{2+}\) may be required for passage of ODV’s through the peritrophic matrix and/or for entering midgut cells via the microvilli. Investigations have focused on identifying proteins of the OB complex that may be involved in the invasion process and may require Mg\(^{2+}\). The ORF CUN085 (222 amino acids, MW of 93 kDa) has been identified as the major occlusion body protein of CuniNPV with no homology to any other known protein. Five major proteins have been identified from purified ODV’s. Experiments are in progress to determine the location and possible interactions of these proteins with the peritrophic matrix and microvilli of host mosquitoes and the role Mg\(^{2+}\) may play in this process.

The epithelial cell surface along the midgut of susceptible and resistant larvae of Anticarsia gemmatalis (Lepidoptera: Noctuidae) to its nucleopolyhedrovirus

Sheila M. Levy\(^1\), Ângela M.F. Falleiros\(^2\), Flávio Moscardi\(^3\) and Elisa A. Gregório\(^4\)

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Anticarsia gemmatalis, is a key pest of soybean in Brazil. It has been controlled by a nucleopolyhedrovirus (AgMPNV), which is widely used as a microbial insecticide in the country. The constant and increasing use of this biological insecticide in some regions has caused concerns about the feasibility of selecting resistant populations. Although this phenomenon has not been detected in the field yet, a laboratory A. gemmatalis population has been selected for high resistance to the AgMPNV. It is known that the midgut is considered one of the most important barriers against viral invasion, before systemic infections can be caused in various tissues if the virus succeeds in reaching the host larva hemocoel. Our work aimed to verify whether or not the AgMPNV invades and infects the midgut cells of resistant larvae, comparing the ultrastructure of the midgut epithelial cells from the susceptible (SL) and resistant (RL) A. gemmatalis infected larvae. The susceptible and resistant strains of A. gemmatalis were reared on artificial diet, under laboratory-controlled conditions at Embrapa, Londrina-PR, Brazil. Fourth instar larvae were used in this study. The midguts of SL and RL were divided in proximal, medial and distal regions, processed and analyzed under transmission and scanning electron microscopes. The proximal midgut regions in the SL presented columnar cells with regular microvilli and apical cytoplasmic projections (smooth to irregular). The medial midgut region in SL showed sparse microvilli and large amount of cytoplasmic projections (usually irregular). The cytoplasmic projections of the distal midgut region in SL were scarce and with membrane disruption. Few microapocrine secretory vesicles were released from the microvilli along the midgut length. The columnar cell surface at the different midgut regions in RL presented an increase in the number of microapocrine secretion as well as in the number of cytoplasmic projections among the microvilli. Many of the cytoplasmic projections exhibited smooth surface with few punctual membrane disruptions. Furthermore, the distal midgut region in RL presented large epithelial infolding with many smooth cytoplasmic projections. Our results show that there are morphological differences in the epithelial wall surface along the midgut of SL and RL of A. gemmatalis, which may be related to the insect resistance to AgMPNV infection. This work has been supported by FAPESP and PRONEX (MCT/Finep/CNPq).

The epithelial cell surface along the midgut of susceptible and resistant larvae of Anticarsia gemmatalis (Lepidoptera: Noctuidae) to its nucleopolyhedrovirus

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midgut cells of RL is still unknown. This work has been supported by FAPESP and PRONEX (MCT/Finep/CNPq).

Poster / Viruses. V-22.

Comparative study on the susceptibility of cutworms (Lepidoptera: Noctuidae) to Agrotis segetum NPV and A. ipsislon NPV

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The common cutworm (Agrotis segetum) and the black cutworm (A. ipsislon) are serious soil pests of many vegetable and field crops all over the world. We have demonstrated the cross-infectivity of two baculviruses, A. segetum nucleopolyhedrovirus (AgNPV) and A. ipsislon nucleopolyhedrovirus (AgNPV) for these two insect pests. The susceptibility of A. segetum to AgipNPV was confirmed by DNA restriction endonuclease analyses of DNA isolated from virus harvested from infected A. segetum larvae. For an initial comparison of both viruses, partial polyhedrin sequences were amplified by PCR, cloned and sequenced. Both viruses shared a very similar polyhedrin gene sequence resulting in only two amino acid substitutions. Phylogenetic analyses clearly demonstrated that both viruses belong to NPV group II and are most closely related to a clade consisting of Spodoptera exigua NPV, S. frugiperda NPV and S. littoralis NPV. Since AgipNPV shows high virulence for both cutworm species, it appears to be a suitable candidate as a single biological control agent of A. segetum and A. ipsislon.

Poster / Viruses. V-23.

Characterization of a truncated chitinase gene within the genome of the Cryptophlebia leucotreta granulovirus

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Viral chitinase (chiA) genes are present in many baculovirus genomes and play an important role in cuticle breakdown and virus release of infected host insects. Recently, the genome sequence of the Cryptophlebia leucotreta granulovirus (CrleGV) was analyzed and a chitinase gene of only 495 nucleotides was identified. Comparison to other baculovirus chitinase genes revealed a large deletion of most of the central coding region including the conserved chitinase active site signature. This finding suggested that the chitinase gene of CrleGV encodes a non-functional enzyme. PCR amplification and sequencing of the entire chitinase gene from different CrleGV isolates from the Cape Verde Islands, the Ivory Coast and South Africa revealed that these isolates also contained a truncated chitinase gene. We performed a functional comparison of the CrleGV chitinase and the chitinase of the Cydia pomonella granulovirus (CpGV), which is closely related to CrleGV and also infective to C. leucotreta. Phenotypic differences of larvae of C. leucotreta infected with CrleGV or CpGV were observed. Larval cadavers, previously infected with CrleGV failed to liquefy, whereas complete liquefication of CpGV infected larval cadavers was observed.


Effects of a protease-expressing recombinant baculovirus on nontarget insect predators of Heliothis virescens

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The baculovirus AcMLF9.ScaLh expresses a basement membrane-degrading protease and represents a new class of recombinant baculovirus insecticides. Risk assessment studies were conducted to investigate potential negative effects of consumption of Heliothis virescens F. larvae infected with AcMLF9.ScaLh, on two common predators, the lacewing Chrysoperla carnea (Stephens), and the ladybird beetle Coleomegilla maculata DeGeer. Predators were reared on one of three feeding regimes consisting of H. virescens larvae that were uninfected or infected with AcMLF9.ScaLh or AcMNPy C6. Control regimes consisted of Siptroga cerealella (Oliver) eggs for C. carnea, and Ostrinia nubilalis (Hübner) eggs and aphids for C. maculata. Survival of C. carnea fed Siptroga eggs and AcMLF9.ScaLh-infected H. virescens was significantly higher than for C. carnea fed H. virescens that were uninfected or infected with AcMNPy C6. There were no significant differences in development time between the two treatment groups. Consumption of H. virescens that were uninfected or infected with AcMNPy C6 or AcMLF9.ScaLh. Baculoviruses ingested by C. carnea larvae remained viable within the digestive tract until adult emergence but had no detrimental effect on egg production. The data suggest that use of AcMLF9.ScaLh in pest management would pose no greater risk to insect predators in the environment than use of the wildtype virus AcMNPy C6.

Poster / Viruses. V-25.

Disintegration of the peritrophic membrane of silkworm (Bombyx mori) larvae due to spindles of an entomopoxvirus

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Mode of action by which entomopoxvirus (EPV) spindles enhance nucleopolyhedrovirus (NPV) infection has been elucidated in the present study. Spindles of Anonoma cuprea entomopoxvirus (Ac-EPV), a coleopteran EPV, are known to enhance Bombyx mori NPV (BmNPV) infection in silkworm (Bombyx mori) larvae. AcEPV spindles were orally administered to silkworm larvae with or without BmNPV polyhedra, and the peritrophic membranes (PMs) were observed using a binocular microscope. Soon after the larvae’s access to spindles with or without the polyhedra had been terminated, some PMs disappeared wholly and some were observed in partial form. Some of the partial PMs observed were very fragile. The disintegration of the PM due to spindles was also observed by the histological sectioning of the midgut. However, a day after the larvae had terminated their access to the spindles, the PM regenerated partially or wholly. In contrast, the administration of AcEPV spheroids caused neither the disintegration of PMs nor the enhancement of BmNPV infection in silkworm larvae. In low vacuum scanning electron microscopic observation on the surface of the PM of larva reared on diet free from spindles and polyhedra, no pores or discontinuities in size that allowed baculovirus virions to penetrate through it were found. These findings strongly suggest that the enhancement of NPV infection occurs due to the attachment of a greater number of NPV virions to the microvilli of cylindrical cells, since spindles lead to the disintegration of the PM as a barrier against NPV virions.


Expression of a Taxoneuron nigriceps polydnavirus (TnBV) encoded protein, TnBV1, is toxic for lepidopteran insect cells

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TnBV is an obligate symbiont associated with the Braconid T. nigriceps, which is a parasitoid of Heliothis virescens larvae. At oviposition, both the parasitoid egg and TnBV are injected into the host, which leads to a disruption of the host immune and endocrine
systems. To identify polydnavirus gene products which contribute to these processes, the expression pattern of TnBV genes from parasitized H. virescens larvae was analyzed and cDNAs of viral transcripts were obtained (Varricchio et al., 1999). One of these viral mRNAs, named TnBV1, was shown to be expressed in prothoracic glands of parasitized host larvae (Varricchio et al., 1999). To study the function of the TnBV1 protein, we attempted to overexpress TnBV1, using the baculovirus Autographa californica nucleopolyhedrovirus (AcMNPV) under the control of the polyhedrin promoter. Isolation of a TnBV1 recombinant virus was dependent upon homologous recombination between the baculovirus Sf91 gal and the transfer vector pSYXIV. Recovery of stable occlusion positive recombinant viruses was not possible, with the exception of recombinant viruses with deletions/mutations within the TnBV1 gene. We hypothesized that TnBV1 expression may be cytotoxic to Spodoptera frugiperda (SF-21) insect cells used to produce the recombinant virus. Therefore, we used the Bac-to-Bac® system to create recombinant baculoviruses maintained in E. coli, which have either TnBV1 or an initiator methionine mutant (ATG-) of TnBV1 cloned under the control of the polyhedrin promoter. Light microscopy examination revealed substantial lysis of SF-21 and High Five™ cells from 48 hours post-infection with the TnBV1 recombinant virus, but not with the TnBV1 (ATG-) recombinant. Budded virus production was unaffected for either recombinant virus compared to wild type (wt) AcMNPV. FACS analysis coupled with a TUNEL assay showed that SF-21 cells infected with the TnBV1 recombinant, but not the TnBV1 (ATG-) recombinant or wt AcMNPV, produced double-stranded breaks in host genomic DNA, indicative of apoptosis. Transient expression of TnBV1 in SF-21 cells, without baculovirus infection, led to a significant decrease in the number of viable cells, which showed that TnBV1 alone can cause these effects. Despite marked effects in cell culture, injection of TnBV1 recombinant budded virus did not result in an alteration of virulence in Heliothis virescens 4th instar larvae compared to wt AcMNPV.

Viral diseases of honey bees are a major concern in apiculture, causing serious losses worldwide, especially in combination with the mite Varroa jacobsoni. The biology of bee viral diseases, their relationship with mites and their transmission among bees are poorly understood. We are investigating the relationship among viruses, mites, and colony decline by examining transmission routes, viral persistence, and mite activation of Kashmir bee virus (KBV), we expressed two KBV structural proteins in the pQE bacterial protein expression system and produced specific, KBV polyclonal antibodies. Virus-specific RT-PCR reactions were developed for both KBV and SBV. In healthy colonies, Kashmir bee virus, and sacbrood virus were detected with co-infection occurring within individual bees. DNA sequencing of both viruses confirmed identifications. RT-PCR was found to be more sensitive. Bees were found to contain detectable viral genome by RT-PCR, but lacked any detectable capsid proteins, indicating that these viruses were truly persistent or latent. In multiple bee colonies of different genotype, both KBV and SBV have been detected in adult bees (workers, drones, queens), eggs, larvae, pupae, brood food, pollen stores, and honey. These data suggest that these viruses can be vertically transmitted and that there is excellent potential for horizontal transmission via the worker secretions into honey, brood food, and pollen stores. Varroa mites also test positive for the viruses and KBV has been detected in mite saliva. This suggests that mites can vector the virus. No colonies tested were found to be virus free. Activation of viruses by mite infestation was tested two different ways and data strongly suggests that mite infestation activates virus levels in a bee. We are currently asking 1) what is the relationship between infection with the viruses and mite infestation levels, and 2) what tissues are infected with viruses.

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DNA polymerase sequence analysis and host range of Ascorvirus isolates from Indonesia and the United States

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Seven isolates from The United States (SC1 isolated from H. virescens, SC2 isolated from H. zeas, SB2-126 isolated from S. frugiperda, SC 3 and SC4 isolated from S. frugiperda in Charleston and Clemson SC respectively) and two isolates from Indonesia (INDO1 and INDO2 isolated from Spodoptera exigua) were compared with respect to the general relatedness of their DNA, host range and morphology. Restriction enzyme analysis showed that the two isolates from Indonesia (INDO 1 and INDO 2) differed from each other and from all other isolates. One isolate from South Carolina (SC 1 from H. virescens) was also unique. The second South Carolina isolate (SC 2 from H. zeas) was the same as a Spodoptera frugiperda isolate (SF -82-126) from Georgia. DNA sequence analysis of the DNA polymerase gene of IND01, INDO2 and SC4 showed that INDO1 and SC4 have 99% similarity with SfAV1, and IND02 has 91% similarity with HvAv3. The translation of the ORF within these IND01 and SC4 sequences that code for DNA-polymerase, showed 96% identity with the amino acid sequence of the DNA-polymerase of SfAV1. The level of similarity between nucleic acid sequences of the DNA polymerase of IND01 and SC4 isolates and SfAV1 suggest that they are closely related. The translation of the ORF within the INDO2 sequence that codes for DNA-polymerase, showed 83% identity with the amino acid sequence of the DNA-polymerase of HvAv3.

Determination of PhopGV activity by a precise surface contamination method

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Phthorimoea operculaella granulovirus (PhopGV) is the main component of viral bio-pesticides used to control the various potato tuber moth (PTM) species. P. operculaella (Zeller) (Lepidoptera: Gelechiidae) is the most widely distributed of them. Several geographical isolates of PhopGV are available but their insecticidal activity against mining PTM larvae has not been precisely evaluated.

We have developed a device to evaluate PhopGV biological activity which is based on aerosol generation with a nebulizer. The device had permitted to obtain a 94% homogeneity and a 97.5% repetitiveness in the dispersion of virus onto the tuber surface. These results improved those generally obtained with other dispersion methods such as Potter tower, which gave less than 60% homogeneity and less than 65% repetitiveness.

A Tunisian isolate of PhopGV was used to test the method. The mortality of P. operculaella larvae obtained with 5 different concentrations (0.6 to 600 granules/mm²) ranged between 10 to 100%. The LC₅₀ was determined to be 9 granules/mm². With this technique, the use of 10⁶ granules permits to cover the tuber surface with the amount of granules needed. This is a considerable decrease in the virus needed for running the experiments, a hundred thousand-fold reduction compared to the immersion technique. As a PhopGV infected larva produces 4 to 5 x 10⁸ granules, a screening of the biological activity can be performed with a single larva.

This spray surface contamination method permits to obtain precise and reproducible results with a limited amount of viral material. It should allow evaluations of the virulence of different isolates of PhopGV against P. operculaella laboratory colonies.
BACTERIA

Endospore degradation in an asporogenic, crystalline mutant of Bacillus thuringiensis

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An asporogenic, crystalline mutant was obtained from the strain HD-73 of Bacillus thuringiensis var. kurstaki. The sporulation process of this mutant start just as it does in the wild-type strain, and forespores are clearly detectable by electron microscopy. However, spore maduration seems to be altered in the mutant as the spore cortex exhibits degradation until the complete depletion of the spore, preceded by the appearance of hollow bodies surrounded by a thin membrane. At the end, only the bipyramidal crystals are observed in the empty cells and cell walls remain, as lysis is deficient. Clearly, the maduration of the spore is severely affected as only 0.2% of the bacteria generate heat resistant spores. A functional SigK factor was detected in the mutant, which is the last active sigma factor during the sporulation process and is required for the expression of genes involved the formation of the spore coat and cortex. An inadequate forespore coat or cortex structure may be the cause for this phenotype.

Poster / Bacteria. B-2. Destruction of bacterial spores by non-contact ultrasound

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Disease-causing microorganisms can be highly virulent even in low numbers and extremely resistant to killing, making it difficult to control human exposure through air delivered mechanisms. Technologies currently available to decontaminate microbes have significant limitations, in part because organisms like Bacillus spores, protozoan cysts, and some viruses are resistant to drying, heat, ultraviolet light, gamma radiation, and many disinfectants. Radiation can be used to destroy bacterial spores, but a large stationary concrete reinforced facility is needed to protect workers and exposure times to hazardous radiation can be lengthy. UV can inactivate microbes on surfaces, but has very limited penetration ability. Sonication can destroy bacterial spores, but it would not be useful for applications in which immersion in water is impractical. Thus, the ability to deal with the threat posed by dangerous airborne microbes has been hampered by limitations of the current technologies. To this effect we investigated the ability of high intensity non-contact ultrasound (NCU) transducers to destroy bacterial spores. NCU surmounts many of these limitations.

Currently, low power ultrasound is widely used for non-destructive evaluation of industrial materials for defect, microstructure, and property characterization, as well as in medical diagnostics for fetus development and tissue analysis. High power ultrasound is used for cell disruption, particle size reduction, vaporization, and can kill bacterial spores (sonication). A common denominator of all conventional applications of ultrasound is that the ultrasound source – the transducer – is physically coupled to the medium to be tested or treated. Generally, the coupling agents are liquids such as water, oils, gels, or grease. Physical coupling is necessary in order to efficiently transmit ultrasound in the materials. Despite the obvious value of ultrasound, applications have been severely stifled by the necessity of physical contact of the transducer to the medium. After many years of R&D, transducers have been produced that generate immense acoustic pressure in air, operating in the frequency range of ~50 kHz to 10 MHz. Using NCU, destruction of 99.99% of dried bacterial spores of a close relative of anthrax, Bacillus thuringiensis was achieved. Following further refinement of the transducers, we anticipate that non-contact ultrasound will have numerous applications including inactivation of agents of bioterrorism and sterilization of medical and surgical equipment, food materials, and air-duct systems of buildings, airplanes, space stations, and others.

Poster / Bacteria. B-3. Laboratory and field experiments for control of Helicoverpa armigera based on bitoxibacillin formulation containing Bt δ-exotoxin Bt

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The Zeraphshan valley is the most important agricultural zone in Uzbekistan. The cotton-growing is the principal branch of the local rural economy. The specific climatic conditions make for the strong activity of the different species of phytophagous arthropods including several species of aphids, cutworm complex, mites and others. The most common and most harmful species is the cotton cutworm-Helicoverpa armigera (Lepidoptera, Noctuidae). At the present time the control of this pest is realized with the different chemical pesticides. This situation creates the seriously ecological problems. We provided preliminary laboratory estimation of toxic activity of the Bt formulation Bitoxibacillin (Russia microbial industry) for H. armigera larvae. It was established that this formulation in concentration from 0.3% provokes 100% pest mortality during 5 days. Based on this laboratory experiments we conducted the field experiments for control of the cotton cutworm. The formulation was applied in the different doses including 1.0, 2.0 and 3.0 kg per hectare. The volume of work suspension was 200 l/ha for each variant. The chemical formulation Phosalone (35% concentration) was used as etalon with dose 2.5 kg/ha. The insect mortality was estimated three times after each 5 days. The technical effectiveness in case the minimal concentration of formulation on the fifth day was 25.1% insect mortality, 37.9% for the middle concentration, and 60.1% for the maximal concentration of formulation; on the tenth day the insect mortality was 29.7%, 58.4% and 65% on the twentieth day, 53.8%, 80.1% and 92.8%, respectively. The insect mortality in the case of the Phosalone did not exceeded 85%. Based on the field experiments we can do the conclusion that Bt formulation with 45 billions of spores, same number of endotoxin crystals and 0.6-0.8% δ-exotoxin can provide control of H. armigera on cotton in climatic condition of the Zeraphshan valley.

The research and development of BT subsp. colmeri strain 15A3 in Tianjin of China

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A high toxicity strain, Bacillus thuringiensis subsp. colmeri strain 15A3 was isolated. It do not produce δ-exotoxin. PCR and RFLP analysis exhibit that the strain contains nine types of ICPs gene : Cry1Aa, Cry1Ac, Cry3Ca, Cry1DD, Cry4A, Cry2Aa, Cry5E, and a kind of Vip genes–Vip3A at least. In this research a specific SDS-PAGE profile was appeared. The strain 15A3 can produce large amount of active insecticidal toxin during large scale (5 or 25 tons) deep tank fermentation at a minimum cost. The potency of liquid culture was about 5000-6000 IU/µg H.a.. Supernatant of the culture also have a high toxic against H.a.. Toxicity of a primary powder reached 50,000 IU/mg H.a.. Wettable powder (16,000-32,000 IU/mg H.a.,) have been manufactured. Both of them showed high toxicity to lepidopteran such as H.a., Spodoptera exigua, Plutella xylostella, Hyphantria cunea, and Lymantria dispar in the field.
Environmental distribution, frequency and diversity of Bacillus thuringiensis isolates from Spain and Latin America

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Bacillus thuringiensis isolates from four collections were analysed with regard to their ecology, crystal morphology, presence of cry and vip genes, and insect toxicity. These collections, obtained from a screening carried out in Spain, Bolivia and Mexico, included 799 strains isolated from agricultural and non-cultivated soil, dust and grains from stored products, water samples and dead insect, among others. A screening for the presence of cry1A, cry2 and vip genes has been performed using PCR. The vip amplicons were digested with restriction enzymes to examine sequence diversity. Variability of size and morphology of crystals, observed under phase contrast microscopy, was described and related to cry gene content as obtained by PCR. Bioassays with Diabrotica virgifera virgifera (Coleoptera), Heliolthis virescens, and Helioconvera zea (Lepidoptera) have been carried out with spore and crystal suspensions, and supernatants from isolates cultures. In most cases, the observed toxicity was in accordance with the PCR and RFLP data.

Diversity of Bacillus thuringiensis strains with insecticidal activity against Lepidopteran and Dipteran insects

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The establishment of programs to search for new combinations of Bacillus thuringiensis genes that express higher insecticidal potency has been shown to be important, since a significant number of pests are not controlled with the available toxins, and because new alternatives to fight resistance are needed. We present the characterization of 457 B. thuringiensis isolates by means of phase-contrast microscopy to check the morphology of the crystal, by means of bioassays with Spodoptera frugiperda (Lepidoptera) and Culex quinquefasciatus (Diptera) larvae to assess the insecticidal activity, by means of PCR with primers for cry1, cry4 and cry11 genes, and by means of sodium dodecyl sulfate polyacrylamide gel electrophoresis. From all 457 isolates, the crystals presented several morphologies: bipyramidal (61%), round (7%), triangular (6.5%), combinations of bipyramidal and round (17.7%), triangular and amorphous (4%), bipyramidal and triangular (1.3%), polymorphic (2%), and amorphous (0.4%). Twelve percent of isolates were active against S. frugiperda and 5.5% against C. quinquefasciatus. The rest (82%) of the isolates did not show any toxicity against the target organisms tested suggesting that they could be toxic to other target insects. In addition, amplification of cry1 genes was obtained in 12.5% isolates. When these isolates were tested with specific primers for each cry1 gene, 36.8% showed the genotype cry1Aa, cry1Ab, cry1Ac and cry1D, 3.5% isolates had cry1Aa, cry1Ab, cry1Ac, and cry1D, 1.7% had cry1Ab, and cry1B, and 1.7% had only cry1Ab. Other genes, such as cry1C, cry1E, cry1F and cry1G were not present in any isolate. The cry1A gene was amplified in 0.65% of the 457 isolates and cry4B in 0.2%. An 8.7% of the isolates did not show any product by PCR, even though they presented toxicity suggesting that they might contain putative new cry genes or some non-tested genes. Different crystal protein profiles of isolates from the same sample showed the great diversity of this bacterium.

Poster / Bacteria. B-6.

Mosquito larvicidity and synergism in transgenic Anabaena expressing four genes from B. thuringiensis subsp. israelensis

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Mosquito larvicidal activity of Bacillus thuringiensis subsp. israelensis (Bti) is contained in parasporal crystal composed of 5 major proteins (of 134, 128, 78, 72 and 27 kDa), encoded respectively by cry4Aa, cry4B, cry10Aa, cry11Aa and cry1Aa, all reside on the 128 kbp plasmid pBTox1 [1]. Three (excluding cry4B and cry10Aa) have been cloned in Escherichia coli together with p20 (encoding an accessory protein) in all 15 possible combinations and express the genes included [2]. Two of these, expressing cry1Aa, p20 and cry4Aa, with or without cry11Aa, display high toxicity against Aedes aegypti larvae.

When all 4 genes were introduced into the nitrogen-fixing, filamentous cyanobacterium Anabaena PCC 7120 for expression under two strong promoters Pnab and Pu [3], it displayed highest toxicity ever achieved in transgenic cyanobacteria against 4th-instar larvae of A. aegypti. Cry1Aa was found to synergize both Cry4Aa and Cry11Aa, and shorten the lethal times (killing quicker), which is why it dramatically reduces the likelihood of resistant development in the target organisms.

The larvicidal activity of *Bacillus thuringiensis* subsp. *israelensis* (Bt) against mosquito and blackfly larvae is included in the following five major polypeptides of the parasporal crystalline body (dendotoxin) produced during sporulation: Cry4A, Cry4B, Cry10A, Cry11A and Cry1Aa (of 134, 128, 78, 72 and 27 kDa, respectively). Cry1Aa is least toxic but is most synergistic to any of the others and their combinations. Cry1Aa demonstrates synergism with heterologous mosquitocidal toxins as well. Its mixture with the binary toxin of *Bacillus sphaericus* is highly toxic to *Culex quinquefasciatus* strain selected for resistance to the latter. In addition, other strains of this mosquito species resistant to single or multiple Cry toxins of Bt retain their original sensitivity levels in the presence of moderate concentrations of Cry1Aa, thus playing a critical role in suppressing resistance to Cry toxins.

Here, we compared the levels of toxicity and synergism of the three known Cry toxins from Bt, Cry1Aa, Cry2Ba and Cry1Ca. Each of the respective cyt was cloned alone in expression vector and overexpressed in *Escherichia coli*. They yield diverse toxicity levels against *Aedes aegypti* larvae and synergism by various combinations of Bt Cry’s expressed in transgenic *E. coli*.

Identification of two isoforms of aminopeptidase N in *Aedes aegypti* larval midgut

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The bacterium *Bacillus thuringiensis* produces toxin inclusions that are deleterious to target insect larvae. These toxins are believed to interact with specific receptor protein(s) present on the gut epithelial cells of the larvae. In various insect species, in particular those belonging to the lepidopteran class, aminopeptidase N (APN) is one of the two receptor proteins that are thought to be involved in toxin-receptor interactions. However, in mosquitoes, the nature and identity of the receptor protein is unknown. Here, using RT-PCR, we have identified two isoforms of APN transcripts in the *Aedes aegypti* mosquito larval midgut. These results are congruent with the previous report of multiple isoforms of APN gene expression in lepidopteran larvae. Which of the two isoforms (or other yet identified receptor proteins) is involved in the killing of mosquito larvae remains to be elucidated.

Comparative studies of *Bacillus thuringiensis* var. *israelensis* growth and spore production in different concentrations of alternative medium

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The successful of bioinsecticide production and commercialization from *Bacillus thuringiensis* var. *israelensis* is given by the culture media utilization, using natural materials, usually low cost industrial by-products. The aim of this study was the comparison of medium concentration (40, 50, 60, 70, 80, 90, 100%) done with manipueira (cassava industrial waste), in order to verify the cellular growth and the spore production. For this, the medium had the pH adjusted to 7 before sterilization, the cellular growth was monitored by optical density (620nm) and COD (chemical oxygen demand) and spore production by pour-plate count during 120 hours. By the absorbance analysis, it was verified the growth is greater as higher is the manipueira concentration. By the pour-plate analysis, it was verified that all medium culture concentrations reach similar spore number, but in different fermentation time. The COD analysis show that 40% (average) of organic matter was consumed by the bacterial growth in all manipueira medium concentrations. Manipueira is considered a pollutant waste due to its high organic charge, what indicates that it could be used as substrate to produce bioinsecticide. Then, it would be convenient to use pure manipueira to reduce the pollutant effect. However, in this study it was observed that as higher the concentration, more slowly is the spore production. That means it wouldn’t recommended to use pure manipueira for this objective. Despite of this, it’s possible to say this use of manipueira is very promising.

Effects of Bt-transgenic potato on *Copidosoma koehleri* a natural enemy of *Phthorimaea operculella*

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The potato tuber moth *Phthorimaea operculella* (Zeller) (PTM) is one of the most damaging potato pests worldwide. Several control components have been identified to manage this pest. Among them, the use of *Bacillus thuringiensis* (Bt) has proved to be effective in reducing PTM infestations in stores. So far, host plant resistance work has not yielded any promising material with appreciable levels of resistance. The expression of Bt genes confers a non-conventional host plant resistance to this pest. The use of biological control by parasitoids is another promising control method. The objective of this study was to determine the effects of Bt-cry1AB5 potato transgenic on the development and reproduction capacity of *Copidosoma koehleri* Blanchard, a PTM endoparasitoid. The studies were made in the biosafety laboratory at the International Potato Center (CIP). PTM eggs were exposed to *C. koehleri* and reared on potato tubers. Totals of 20,000 and 6000 PTM eggs were used from each of the two treatments, i.e., Bt tuber and non-Bt tuber (control) respectively, with three repetitions in a complete randomized design. After completion of the larval development, the number of pupae and mummies were recorded, and they were weighed, and measured. *C. koehleri* mummies were placed in a small tube for emergence. The adults were reared in pairs and exposed to 15 PTM eggs in order to evaluate oviposition capacity. The biological parameters evaluated were larval and pupal time of development, sex ratio, number of adults per mummy and longevity. Bt plants fed to PTM larvae had a significant effect on the duration of *C. koehleri* mummy development, their weight (8.83 ± 2.71 mg and 12.33 ± 1.76 mg for Bt-tuber and non-Bt-tuber respectively), length (0.89 ± 0.13 mm compared to 1.00 ± 0.08 mm) and adult *C. koehleri* number. The duration of egg development was not significantly affected; nevertheless, the range of number of days in the Bt-tuber was from 14 to 58 days as compared with 18 to 28 of the control. The sex ratio increased in favor of males (3.94 and 1.59 on Bt and non-Bt-tubers respectively). Regarding the progeny obtained from the individuals reared on Bt tubers, there was no effect on reproduction capacity (63.20 and 71.75 eggs in both treatments) while the longevity was significantly different compared with that on the non-Bt tubers. These results demonstrate that the expression of Bt-cry1AB5 can have some impact on the development of *C. koehleri*. This needs to be taken into account in the case when these two promising PTM management options are considered in an IPM strategy.

Suitability of Genetically Modified *Bacillus thuringiensis* WG-001 for safety release on cotton fields

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To evaluate the suitability and feasibility of genetically modified *Bacillus thuringiensis* strains in natural environment, *Bacillus thuringiensis* WG-001 expressing Cry1Aa and Cry1Ac as the molecular
tracer was applied to the cotton phylloplane by spray at Baoding area of Hebei province, China. The strain WG-001 was screened by the plate counts of serially diluted bacterial suspension and identified by the PCR amplification of tracser gene from the soil and the leaf surface. The aerial dispersal distance from the spray area was monitored with 3.5-cm-diameter petri dishes that were placed on the ground in a spokelike formation around the experiment field at a distance of 5m, 10m, 15m. To assess the impact of field release WG-001 on indigenous microbes, soil samples were taken from the spray area at the section of 3.5-cm below the soil surface. Then 10-fold series dilution soil suspension was prepared and aliquots of appropriate dilutions were plated on different media for quantitative analysis of the total populations of fungi and bacteria. We found that the range of fifteen-meter in downwind direction and five-meter in upward direction is the maximum dispersal area. Concentration of WG-001 on cotton leaves decreased continuously and no WG-001 colony could be examined after 35 days. In addition, the vertical dissemination depth of WG-001 in soil is no more than 5-cm in the spray area. The total amount of indigenous bacteri populations fluctuated at the range of 3.9 × 10⁶ cfu/g soil and 4.2×10⁶ cfu/g soil and the range of the total numbers of indigenous fungi populations is 1.2 × 10⁶ cfu/g soil to 1.7 × 10⁶ cfu/g soil. As a result, there is a restriction of the diffuse distance by airstream and survivability on cotton leaves of the strain WG-001, and no significant impacts on indigenous microbial populations were found in soil after WG-001 was applied. *This research was supported by HiTech Development Project of China ("863" Project) [Funds No.2001AA212301] and the National Natural Science Foundation of China (No.30170032)

Poster / Bacteria. B-14.

Identification of the aminopeptidase N carbohydrate binding determinant for Bacillus thuringiensis Cry1Ac toxin

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Bacillus thuringiensis Cry1Ac toxin is highly toxic to the leipodopteron, Manduca sexta. This insect has been invaluable as a model for investigating Cry1 toxin mode-of-action. Protease-activated Cry1Ac toxin receptors in the midgut epithelium, undergoes a conformational change and inserts into membrane creating water-filled pores. Cry1Ac binds multiple proteins in M. sexta midgut including a cadherin-like protein, an aminopeptidase N (APN), and alkaline phosphatase. Cry1Ac recognizes an N-acetyl-galactosamine (GalNAc) moiety on a specific APN, called MsAPN1. Preliminary studies by several research groups indicate that an oligosaccharide containing GalNAc is involved in the recognition process. MsAPN1 was purified from brush border membrane vesicles (BBMV) isolated from M. sexta using anion exchange chromatography, separating both soluble (115 kDa) and glycosylphosphatidylinositol (GPI) anchored (120 kDa) forms. These proteins were resolved on SDS PAGE and then subjected to blotting assays with Cry1Ac and various lectins. Both forms were strongly recognized by biotinylated Cry1Ac. Concanavalin A (ConA), Dolichos biflorus agglutinin (DBA), soybean agglutinin (SBA), Arctopus integrifolia (jacalin), Rcinus communis (RCA), Ulex europeus agglutinin (UEA), Maclura pomifera lectin (MPL) recognized the 120 kDa form. ConA, DBA, and UEA recognized the 115 kDa form, suggesting that although some glycan residues are lost upon cleavage of the GPI anchor, lectins that recognize GalNAc and related sugars remain on the 115 kDa form after solubilization. **This research was supported by the National Health and Medical Research Council of Australia [grant number APP1035287].

We previously showed that increased levels of Cry1Ac resistance in the YHD2 strain of H. virescens after continuous selection with this toxin correlated with reduced toxin binding and changes in midgut protein glycosylation. More specifically, soybean agglutinin (SBA) recognition of glycoproteins of 68- and 63-kDa was reduced in brush border membrane vesicles (BBMV) from YHD2 larvae when compared to BBVMM from susceptible (YDK) or the offspring (F1) of a backcross between YDK and YHD2 adults. Because both BBMV from YDK and F1 larvae bound Cry1Ac similarly, these results were evidence for a correlation between altered glycosylation and reduced Cry1Ac binding. Since SBA binds to terminal N-Acetylgalactosamine (GalNAc) residues, and Cry1Ac recognizes this carbohydrate in both BMMV, absence of this sugar in toxin binding glycoproteins from BMMV of YHD2 insects may account for decreased Cry1Ac binding to these vesicles. Our main goal was to identify the proteins with altered glycosylation in BMMV from YHD2 and study their role in Cry1Ac mode of action. Competition of SBA binding to the 63- and 68-kDa BMMV proteins on blots was greatly inhibited by the presence of Cry1Ac, suggesting binding of Cry1Ac to both BMMV glycoproteins. SBA binding was not affected by Cry1Ac mutant QNR313. AAA, which lacks a GalNAc binding pocket, demonstrating that Cry1Ac was blocking SBA binding by recognition of a
GalNAc residue. This was also demonstrated by elimination of glycans from BBMV proteins on blots by treatment with sodium periodate or specific glycosidases. Further work focused on the 68-kDa glycoprotein, resulted in its identification and characterization of the glycans moieties recognized by Cry1Ac. The role of this glycoprotein in Cry1Ac mode of action and resistance in YHD2 larvae will also be addressed.

Poster / Bacteria. B-17.  
**Mapping the receptor binding sites on Bacillus thuringiensis Cry1Aa toxin using blocking molecules**  
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The insecticidal specificity of Cry toxin seems to be largely dependent on receptor recognition. In *Bombyx mori*, aminopeptidase N (BmAPlN) and cadherin-like protein (BrTr75) have been identified as Cry1Aa toxin receptors. Identifying the binding sites involved in the Cry1Aa toxin-receptor interactions could provide us a key perspective on the mechanism of insecticidal specificity, which could form a basis for improving the specificity and toxicity of Cry toxins.

To avoid misinterpretations that might result from the unexpected effects of point mutations introduced outside the binding site, we analyzed receptor-binding sites using two methods that introduced blocking molecules on the surface of Cry1Aa toxin.

Of seven monoclonal antibodies constructed against Cry1Aa toxin, 1B10 and 2C2 inhibited the binding of Cry1Aa toxin to BmAPlN1, suggesting that their binding sites (epitopes) are located close to the BmAPlN1 binding site of the toxin. To identify the BmAPlN1 binding site on Cry1Aa, we first analyzed the epitopes of those two antibodies using Cry1Aa deletion mutants and synthesized peptides. Then, two candidate epitopes were determined: one site consisted of 507-512 and 582-589 and the other site consisted of 520-527 and 570-577. To determine the true epitopes of the antibodies, cysteine substitutions were introduced at 521Arg or 582Val on Cry1Aa and then a smaller blocking molecule, N-(9-acridinyl)maleimide (NAM), was covalently bound to the -SH of Arg521Cys and Val582Cys. The binding assay showed that both blocking antibodies bound the Val582Cys toxin, but not the Val582Cys-NAM toxin, suggesting that the epitopes of the two antibodies were located adjacent to the Val582Cys of Domain III. In addition, NAM covalently bound to Val582Cys affected BmAPlN1 binding to the Val582Cys toxin, but not BrTr75 binding to it, and reduced the toxicity of Val582Cys toxin in *Bombyx mori* larvae. These results suggest that the BmAPlN1 binding site on Cry1Aa is located near 582Val and that BmAPlN1 functions as a receptor for Cry1Aa toxin in *Bombyx mori* larvae. Currently, we are analyzing the BrTr75 binding site on Cry1Aa using the same methods.

Poster / Bacteria. B-18.  
**The chymotrypsin mutants of Bacillus thuringiensis Cry1Aa toxin: planar lipid bilayer and light scattering analyses, interaction with Manduca sexta midgut receptors**  
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The *Bacillus thuringiensis* Cry1Aa [epsilon]-endotoxin is an insecticidal crystal protein, with a molecular mass of 130 kDa. To become toxic, the protoxin is first solubilized then activated to a 65 kDa toxin core by insect midgut proteases. However, insufficient processing or overdigestion of the activated toxin may reduce its bioactivity or render it completely inactive. Upon binding to specific receptors in the midgut of susceptible insects, the activated toxin exerts its insecticidal activity. This activity is correlated with the toxin’s capability to integrate into the membrane of the insect gut and subsequently kill the insect. The overall objective of the present work was to create a more stable protease resistant Cry1Aa toxin core by mutating potential chymotrypsin and trypsin sites of the toxin. To that end, native cry1Aa gene was cloned and expressed on a plasmid, pBA1, which was used as a template to create mutants by site directed mutagenesis. Tests were performed on the ion channel and pore formation capability of the Cry1Aa chymotrypsin mutants in planar lipid bilayers (PLB) and vesicle swelling assays by using brush border membrane vesicles of *Manduca sexta*. Compared to wild type, the *in vitro* protease assays showed no enhanced stability differences between mutant and wildtype Cry1Aa toxins. Light scattering assays indicated a differential range of kinetic pore formation between the Cry1Aa wild type and mutant toxins. One unexpected finding was that removal of the protease cleavage site at amino acid position R28 of Cry1Aa toxin did not suppress pore formation.

**STU** Poster / Bacteria. B-19.  
**Proline substitution in [4] affects helical hairpin-flexibility and membrane perturbation of the Bacillus thuringiensis Cry4B toxin**  
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The [4–5] hairpin of the *Bacillus thuringiensis* Cry [epsilon]-endotoxins has been proposed to be involved in formation of a lytic pore in the midgut cell membranes of susceptible insect larvae. In this study, effects of single proline substitution at Gln-149 located near the center of [4] of the Cry4B mosquito-larvicidal protein were investigated. Toxin inclusions of Q149P produced in *Escherichia coli* showed a drastic decrease in toxicity against *Aedes aegypti* larvae, whilst that of Q149A still retained high larvalcidal activity. Additionally, the 65-kDa trypsin-treated Q149P toxin (ca. 40mM to 380 mM) was still able to perturb liposome vesicles in calcine-release assays, but displayed much lower activity compared to the wild type and Q149A toxins. Furthermore, molecular dynamics simulations of the [4–5] hairpin in a POPC/water system revealed that the proline-induced kink in [4] at Gln-149 significantly decreased the flexibility of helices 4 and 5. These results suggested that the flexibility of the [4–5] hairpin is important for larvicidal activity of the Cry4B toxin, possibly in forming a functional toxin-induced pore.

**STU** Poster / Bacteria. B-20.  
**Characterization of the cloned Cry4B domain III fragment**  
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Various investigations have shown that the C-terminal domain III of *Bacillus thuringiensis* Cry [epsilon]-endotoxins is being involved in structural integrity, receptor binding, insect specificity or ion-channel regulation. In this study, the cloned domain III of Cry4B [epsilon]-endotoxin was over-expressed as a soluble form in *Escherichia coli* upon IPTG induction. However, *E. coli* cells expressing the domain III protein showed no toxicity against *Aedes aegypti* mosquito larvac. Circular dichroism spectroscopy indicated that the 23-kDa domain III fragment exists as a [epsilon]-sheet structure. By using surface plasmon resonance biosensor, the cloned domain III protein was shown to bind irreversibly to immobilized PE/PC/CH bilayer membranes similar to the 65-kDa activated Cry4B toxin. In addition, treatment with proteinase K showed a reduction of the binding-sensing signal of both the domain III and the full-length Cry4B toxin, but still retained the signal that is significantly higher than the baseline. These results indicated that the proteins bound and inserted into the lipid membrane, and therefore suggested that the domain III region is involved in membrane binding and insertion.
Poster / Bacteria. B-21.

Mobility of plasmid-borne genes encoding disease of a New Zealand scarab pest, *Costelytra zealandica*

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Amber disease of the New Zealand grass grub, *Costelytra zealandica* (Coleoptera: Scarabaeidae), is caused by some strains of *Serratia entomophila* or *S. proteamaculans* (Enterobacteriaceae). Virulent strains of these two species contain large plasmids which carry the disease-encoding genes. Under *in vitro* conditions, the disease-encoding plasmids can move between the strains of the two species. In soil microcosm experiments, plasmid transfer could be detected in sterile and nutrient-amended natural soil, but not in natural soil. However, when grass grub larvae were present in the natural soil, plasmid transfer was detected at levels equivalent or higher to those seen *in vitro* (up to $10^6$ transconjugants/recipient), indicating the importance of the insect as a niche for horizontal gene transfer in the environment. No other *Serratia* species have been shown to cause amber disease in nature, but disease-encoding plasmids were transferred to *S. marcescens*, *S. ficaria* and *S. liquefaciens* *in vitro* and, in some cases, recipients of the plasmids were able to cause disease.

The three plasmid-borne genes encoding amber disease (*sepA, sepB and sepC*) are essential for disease and encode proteins which have high similarity to the recently described family of proteins known as the Tc family. Genes encoding Tc proteins have previously been described from *Photobacterium luminescens*, *Xenorhabdus nematophila* and *Vesinia pestis*. A strain of *Vesinia frederiksenii*, isolated from a grass grub larva, was found to contain three genes, two of which had 90% DNA similarity to the *sepAB* genes. This finding, together with the analysis of several other *Serratia* plasmids which lacked the disease-encoding genes, suggests that the virulence-encoding genes may be functioning as a horizontally mobile pathogenicity island. Sequencing has identified several elements associated with gene mobility on the borders of the potential island, further supporting this hypothesis. Our studies indicate that plasmid transfer between *Serratia* strains and species has the potential to influence the epidemiology of amber disease. Horizontal gene transfer appears to play an important role in the evolution and spread of insecticidal toxin genes in bacteria.

Poster / Bacteria. B-22.

Maximizing the use of mass spectrometry data generated from proteomic analyses of insects with relatively few sequenced proteins

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Proteomic analyses are used to examine processes in cells through large-scale examinations of proteins in specific contexts. Generally combined with two-dimensional electrophoresis (2DE), mass spectrometry is a potentially powerful tool used to identify proteins of interest. Proteins are enzymatically digested into peptides that are analyzed by mass spectrometry generating spectra that represent the peptide mass fingerprint (PMF) of the digested protein. Using web-based programs, these fingerprints are compared to theoretically digested peptides of database entries and potential protein identifications are obtained. A probability score is assigned providing an estimation of confidence that identifications are correct. As proteomics becomes more widely used in studies of various organisms, the number of protein sequences in databases has become a limiting factor in utilizing the power of mass spectrometry. Organisms without sequenced genomes, or those that are not widely studied, such as *Lepidoptera*, are underrepresented in protein databases. This may prevent accurate identifications, or high probability identifications, of proteins as the number of potential non-matching proteins (i.e., from different organisms) will be much larger than the number of proteins from the organism of interest. Another contributing challenge is that database sequences consist of protein sequence alone and have none of the post-translational modifications found *in vivo*. However, when a spot from a 2D gel is digested and analyzed by mass spectrometry, the resulting spectra will represent peptides with post-translational modifications. By searching against peptides without these modifications, mass mismatches will occur which lowers the probability of correct identifications.

To maximize the utility of the data generated by mass spectrometry, a combination of further analyses is crucial. Complimentary techniques, such as Western blotting, and in depth analysis of peptide sequences can greatly increase the confidence of correct protein identifications.

Poster / Bacteria. B-23.

Analysis of midgut brush border proteins in *Bt* susceptible and resistant *Plutella xylostella* larvae using differential two-dimensional electrophoresis

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The diamondback moth, *Plutella xylostella*, is the only insect known to have field populations resistant to *Bacillus thuringiensis* (*Bt*). Although these insects typically have only been exposed to preparations of *Bt* kurstaki (containing Cry1Aa, Cry1Ab, Cry1Ac, and Cry2A), field-resistant colonies have also reduced susceptibility to Cry1Ja and Cry1Fa toxins. Numerous studies have demonstrated a reduction in Cry1A binding sites using *in vitro* binding assays suggesting that elimination or alteration of a binding receptor is responsible for resistance. With a resistant colony from Hawaii, however, Biacore studies demonstrated that there is only a slight reduction in the number of binding molecules in resistant *P. xylostella*. Additionally, a 120 kDa aminopeptidase N was found at similar levels and capable of binding Cry1A in both susceptible and resistant insects. Identification of the molecule(s) responsible for resistance has remained elusive. Genetic linkage analysis of isozyme polymorphisms of a Philippine-derived colony of resistant *P. xylostella* revealed a strong correlation between Cry1A resistance and mannose-6-phosphate isomerase. Yet no genes or proteins have been conclusively identified as being responsible for resistance.

To try to identify proteins that are up- or down-regulated in resistant *P. xylostella* versus susceptible, we used a proteomics approach to compare midgut protein patterns. Global differences between the midgut brush border proteins of both strains of *P. xylostella* were examined using fluorescent dyes and two-dimensional electrophoresis. Protein spots of interest were excised from gels and subjected to mass spectrometry resulting in a peptide mass fingerprint (PMF) for each protein analyzed. The protein database at NCBI was searched using the PMF data resulting in potential identifications. Low probability matches and narrowing of the list of candidate identifications was accomplished through peptide sequence analysis and Western blotting.


Interaction of *Bacillus thuringiensis* toxins with *Helicoverpa armigera* midguts

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*Bacillus thuringiensis* Cry1Ab and Cry1Ac toxins share 86% of their amino acidic sequence, however, they produce different levels of toxicity in certain lepidopteran species. This case has been reported for *Helicoverpa armigera*, which is more susceptible to Cry1A than Cry1Ab. Difference of toxicity may rely on subtle differences in any step of the mode of action of these toxins. A candidate step is binding of toxins to midgut membrane receptors since it has been proposed as a key step in the process.

Binding experiments of activated Cry1Ab and Cry1Ac toxins with brush border membrane vesicles (BBMV) from last instar larvae midguts of *H. armigera* showed that both toxins bind with similar
affinity and compete for common sites. Further characterization of binding sites was performed with inhibition binding experiments. Prior to binding, we performed different assays: i) 125I-labelled toxins were pre-incubated with one of 4 different sugars or, ii) BBMV were pre-incubated with one of the 3 lectins that specifically bound to the assayed sugars. In contrast to Cry1Ab binding, Cry1Ac binding was highly inhibited by N-acetylgalactosamine and N-acetylneuraminic acid and the lectin soybean agglutinin. Other sugars, mannos and N-acetylgalactosamine, and the lectin wheat germ agglutinin, had little inhibition binding effects for both toxins, in contrast, concavalin A lectin (which binds mannos) strongly inhibited binding of both toxins.

Poster / Bacteria. B-25.
Identification of the western spruce budworm midgut receptor for Bacillus thuringiensis insecticidal Cry toxins
Aligmantas P. Valairis
USDA Forest Service, Northeastern Research Station, Delaware, OH 43015, USA

The mode of action of Bacillus thuringiensis Cry toxins, which results in cell lysis and larval death, involves proteolytic activation of the protoxins (pro-Cry) and lepidopteran gut-binding of the toxins to receptors on the brush border membrane of gut epithelial cells followed by their insertion into the membrane. Early studies have shown that the presence of toxin-specific binding receptors is essential for insecticidal action. Aminopeptidase N (APN) and cadherin-like proteins from several insects were identified as putative receptors for B. thuringiensis toxins based on binding, the disruption of gene expression, and the expression of target proteins in heterologous expression systems. A different toxin-binding molecule that stained blue with the calcium mimic dye using Stains-all was identified in the brush border membrane of Lymantria dispar. Although these studies have revealed their relationship with toxin susceptibility, further studies are needed to define the functional role of these toxin-binding molecules in the insecticidal activity of B. thuringiensis.

In this study, a novel Bt toxin receptor was identified in the western spruce budworm, Choristoneura occidentalis. Evaluation of its toxin-binding properties by ligand-blotting assay revealed that it binds a number of toxins that are known to be lethal to the budworm. This toxin-binding molecule, BTR-100, displays an apparent size of approximately 100 kDa, is sensitive to degradation with proteases, and appears to be glycosylated. It stains blue with Stains-all, indicating that it may be an anionic calcium-binding protein. Based on homology of its N-terminal amino acid sequence to a yolk-degrading protease previously characterized in Bombyx mori, BTR-100 was tentatively identified as an elastase-like protein. This finding is consistent with the observation that BTR-100 stains blue with Stains-all, as is characteristic of serine proteases, which are known to have Ca2+ binding sites that interact with the calcium mimetic dye. Moreover, the identification of BTR-100 as a protease implies that after binding, the Bt toxin receptor proteolytically triggers the formation of an oligomeric pre-pore structure leading to the irreversible insertion of the toxin into the brush border membrane of midgut epithelial cells that causes lysis of the cells and death to the insect.

Wolbachia in sucking lice
George Kyei-Poku, Doug D. Colwell, Paul Coghill and Kevin D. Floate
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Study of the associations between symbiotic bacteria and parasitic arthropods continues to elucidate an increasingly complex suite of relationships. Sucking lice (Insecta: Anoplura) depend on symbiotic bacteria that provide essential nutrients to supplement their blood diet. These bacteria have now been characterized as members of a group of gamma-Proteobacteria found in a variety of insects. Other symbiotic bacteria, particularly the rickettsia-like genus Wolbachia are being reported from increasing numbers of insect taxa where their role in cytoplasmic incompatibility, parthenogenesis induction, male killing, feminization and overall sex ratio distortion has received a great deal of attention. PCR amplification of nine species of sucking lice using the wsp gene primer set determined the presence of Wolbachia. Detailed sequencing information allowed the construction of a phylogenetic tree relating the isolates from the various louse species. Sequencing also established that each louse species harbors two or more strains of Wolbachia. Co-occurrence of at least two symbiotic bacteria in lice opens several questions regarding their role in louse reproduction and in the vector competence of various louse species.

Adenylyl cyclase and protein kinase A affected the hemocytes-mediated responses of Malacosoma disstria to Xenorhabdus nematophila and Bacillus subtilis
Vladislav Gulij, Cory L. Brooks and Gary B. Dunphy
Dept. of Natural Resource Sciences, McGill Univ., Montreal, Quebec, Canada

The pest insect forest tent caterpillar, Malacosoma disstria has two types of hemocytes involved in antibacterial responses, the granular cells and plasmatocytes. Both of which bind to glass slides and bacteria. Both antigens type elicited signal transduction leading to the immune response. The secondary messenger, cyclic AMP, affect hemocyte adhesion to the slides. forskolin, an activator of adenylyl cyclase decreased the adhesion of both granular cells and plasmatocytes to slides. Inhibiting the enzyme with 9-(Tetrahydro-2’-furyl) adenine and MDL-12,330A, Hydrochloride decreased granular cells adhesion and increased granular cell adhesion, respectively. Plasmatocytes activity was not affected by either inhibitor. Etazolate, a phosphodiesterase inhibitor, increased granular cells attachment but not plasmatocytes adhesion.

The effect of these compounds on the adhesion of entomopatho-
genic gram-negative Xenorhabdus nematophila and nonpathogenic gram-positive Bacillus subtilis to the both hemocytes types is discussed in terms of hemocytes responses to glass slides and bacterial removal from the hemolymph.

Cyclic AMP modulates the activity of protein kinase A (PKA). The PKA inhibitor, Rp-8Br-cAMP increased the level of plasmatocytes and granular cells with Xenorhabdus nematophila but the activator Sp-8Br-cAMP did not affect the hemocytes responses. The extent of hemocytes binding to Bacillus subtilis, in contrast, was decreased by Sp-8-Br-cAMP. Rp-8-Br-cAMP increased bacterial-hemocyte contact. Similarly, both drugs modified the removal of Bacillus subtilis and Xenorhabdus nematophila from the hemolymph in vivo.
Immo-dot blot and Western blotting of the hemocytes lysate revealed an 81 kDa that reacted with antibody to human Toll like receptor 2 and 100 kDa protein binding antihuman Toll like receptor 4 antibody. The relationship of these proteins with cyclic AMP levels is presented.

**STU Poster / Bacteria. B-29.**

*Xenorhabdus toxins: novel bacterial insecticides*

Laura Baxter¹,², Alan Morgan¹, Paul Jarrett¹ and Craig Winstanley²

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Bacterial toxins, such as those from Bacillus thuringiensis (Bt), have been used successfully for many years as insecticides. Constraints with the use of Bt include a limited range of target pests and the evolution of insect resistance, meaning that alternatives are highly sought. Recently discovered toxins from *Xenorhabdus* species offer a realistic replacement or complement to existing insecticides.

Our studies are aimed at identifying and characterising novel insecticidal toxins from *Xenorhabdus* species, and examining their mode of action. We have identified, sequenced and cloned five insecticidal toxin genes and a chitinase gene from *X. nematophilus* PMF1296. Other *Xenorhabdus* strains were isolated from soil samples, and their toxic activity against two orders of insect assessed. A study of the toxin gene diversity within over 400 strains showed that the five toxin genes were widely dispersed throughout the collection, and that they are present in both our insect active and inactive strains. DNA sequencing and analysis of a 13kb region outside of the known genes was performed. This identified two new genes and highlighted that the DNA region represented a pathogenicity island on the chromosome. The mode of action of these toxins is being studied. Results from light-scattering assays so far indicate that they are not acting through pore formation, the established mode of action of Bt toxins. However, in vitro binding assays reveal that there is some interaction between the toxin and both cultured insect cells and insect midgut brush border membrane vesicles. The protein toxin does not appear to need processing, and the proteins involved in this interaction are being studied in more detail. These results are encouraging, as new toxins with novel modes of action are urgently required as alternatives to Bt for use in pest control. They may also be effective against those insects that have already become resistant to Bt toxins.

**STU Poster / Bacteria. B-30.**

*Endoparasitic nematodes as targets of nematicidal Bt crystal proteins in transgenic plants*

Xiangqian Li, Sourouh Parsa, Raffi V Aroian

Section of Cell Development Biology, Univ. of California, San Diego, CA 92039-0349, USA

Plant-parasitic nematodes (PPNs) cause an estimated annual economic loss of $100 billion worldwide. Currently, the control of PPNs relies on crop rotation, resistant varieties and chemical application, although, given the amount of damage caused by PPNs, these control techniques are not completely adequate. The main chemical used in nematode control, methyl bromide, faces imminent prohibition on January 1, 2005.

*Bacillus thuringiensis* (Bt) crystal proteins have been presented as an environmentally-friendly alternative to chemical pesticides for the control of insect pests. Our laboratory has further confirmed that four Cry proteins (Cry 5B, Cry 6A, Cry1A, Cry2A) are toxic to phylogenetically diverse free-living nematodes. These results raise the possibility that Cry proteins may hold potential in controlling plant-parasitic nematodes as well. Our goal is to test the hypothesis that PPNs are targets of *Bt* crystal toxins. To begin with, we are testing this hypothesis with Cry6A toxin.

Since the most damaging PPNs are obligate endoparasites that feed and develop from inside the plant root, to effectively test our hypothesis, we must express the Cry proteins in the plant root where the nematode will be able to feed on them. It is well established that the wild-type bacterial genes are poorly expressed in transgenic plants. This poor expression is due to the fact that bacterial genes often contain sequences interpreted by the plant as polyadenylation sites, introns, or a signal for mRNA destabilization. We have synthesized a “plant-friendly” version of cry 6A (1425 ntd) by eliminating sequences predicted to cause post transcriptional gene silencing. In order to test if these factors caused gene silencing and to increase level of expression, this gene driven by 2XCaMV35S promoter was transformed into tomato root and Arabidopsis plants using *Agrobacterium rhizogenes* and *A. tumefaciens* transformation system, respectively. Although some expression has been seen, we are currently troubleshooting to maximize expression. Our progress on Cry6A expression in transgenic plants and potential testing for control of PPNs will be reported.

**Poster / Bacteria. B-31.**

*Bacterial male-killers: inherited symbionts with a cut-throat strategy*

Michael E. N. Majerus¹ and Helen E. Roy²

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Cytoplasmically inherited endosymbionts, such as *Wolbachia*, are known to use four manipulative strategies to promote their spread: cytoplasmic incompatibility, parthenogenesis induction, feminisation and male-killling. Within the bacteria, three of these strategies appear on current evidence to be mainly the province of *Wolbachia*. The exception is male-killling which is known from a diverse array of bacteria, suggesting that this may be the most easily evolved strategy. Male-killers have been reported from a variety of insect orders, with some groups (ladybird beetles, milkweed bugs, nymphalid butterflies) being hotspots for attack.

The dynamics of male-killling endosymbionts depends on factors such as prevalence, vertical transmission efficiency, horizontal transmission, cost to infected females and sources of fitness compensation. Estimates of these parameters, from empirical evidence will be reviewed. Correlations between these parameters and population sex ratios will be described. Case studies in which empirical evidence appears to be at variance with theoretical predictions will be briefly considered. Consequences of female biases in population sex ratios on the evolution of host reproductive strategies, including intra-genomic conflict, changes in mating preferences, investment in copulation and sex role reversal will be discussed. Areas warranting further investigation will be highlighted.

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

*Microbial control of social insects*

Symposium. Wednesday, 8:00.

*Disease resistance vs. biological control of social insects. And the winner is...*

Rebeca B. Rosengaus

Dept. of Biology, Northeastern Univ., 414 Mugra Life Sciences Bldg., Boston, MA 02115-5000, USA

Social insects, particularly ants and termites, live under important pathogenic constraints. They nest, feed and/or forage in environments that support high microbial activity, including pathogenic microorganisms such as bacteria, fungi, nematodes, viruses, etc. The impact of disease and parasitism within a colony can be exacerbated by the frequent and close-range social interactions among nestmates and their ability to control microclimatic conditions in the nest. Yet, in spite the high risks of infection, ants and termites thrive within their microbe-rich environments because they use behavioral, biochemical and immunological mechanisms to reduce the risks of infection. Furthermore, many of these adaptations can be socially
and field studies. Social interaction and grooming can aid the spread susceptibility of termites to these fungi. The difference between per-hostile to entomogenous fungi, and needs to so because of the high activity in the mid-90’s has considerably quietened down.

Biological Termiticide) that was registered by the US-EPA and resulted in at least two patents and at least one product (Bio-Blast® Metarhizium anisoplie range of isolates of the major entomogenous fungi, particularly social interactions. They are also extremely susceptible to a wide mycostatic activities.

Termites are considered as good candidates for control with patho- gens because they live in a conducive environment–humid, minimal diurnal temperature fluctuations, crowded and with considerable social interactions. They are also extremely susceptible to a wide range of isolates of the major entomogenous fungi, particularly Metarhizium anisoplie and Beauveria bassiana. These findings and beliefs led to considerable research in the 1980’s and 90’s which resulted in at least two patents and at least one product (Bio-Blast® Biological Termiticide) that was registered by the US-EPA and was sold and marketed in the USA and Japan. Entomopathogenic fungi are not available commercially today, and the flurry of research activity in the mid-90’s has considerably quietened down.

Termites actually live in an environment which is extremely hostile to entomogenous fungi, and needs to be so because of the high susceptibility of termites to these fungi. The difference between perception and reality is often the difference between laboratory studies and field studies. Social interaction and grooming can aid the spread of conidia from a dosed individual to others, but grooming also removes conidia from individuals and most likely triggers an alarm response in the colony. Infected workers and soldiers appear to remove themselves from the colony and in other field situations, infected individuals are forcibly ejected or walled-off from the rest of the colony. The control of disease spread within the colony, the apparent inability of the fungi to grow in the soils of mounds, nests and galleries, and nest temperatures which can reach 37°C in some colonies make it very difficult for an epizootic to be established.

Fungi can be used now to support other termite control measures. Spot treatment of active feeding sites within a structure is potentially a better alternative to injection of Arsenic Trioxide or Chlorpyrifos and can be used in conjunction with chemical barriers or external baiting techniques. The secret, subterranean and multi-feeding site habits of a termite colony provides considerable protection against infiltration of the ‘bunker’ by a pest control operator armed with a ‘tank’ of Metarhizium. Applications of the fungus can only be made at identified sites which may represent too small a proportion of the colony to initiate a fatal epizootic. Failure to control termite colonies in the field is easy to show, successful control is very difficult to show. Even though methods such as ‘mark-release-recapture’ have been shown to have some problems, methods such as this need to be developed and utilised in order to quantify the impact of fungal treatments on field colonies.

Symposium. Wednesday, 8:30. Studies on the resistance mechanisms exhibited by the eastern subterranean termite Reticulitermes flavipes Drion Boucias and Verena Blaeske Dept. of Entomology & Nematology, Univ. of Florida, Gainesville, USA

Soil-dwelling termites inhabit an environment that is well-suited for colonization by insect mycopathogens. In the subterranean environ- ment, the infectious propagules are buffered from detrimental fluctu- ations in humidity and temperature and from exposure to sunlight. Significantly, the microclimate of the termite colony is humid, a requisite for the survival and development of insect fungi. Lastly, termites, being social insects, live in high densities and individuals come into contact frequently. Many of the mycopathogens are soil- dwellers and are defined as density-dependent agents requiring high humidity for infection. However, in nature, natural epizootics in ter- mites are rare; the majority of the reports concerning termite diseases are from aging termite colonies held in laboratories. Preliminary bioassays in our laboratory with randomly selected mycopathogens demonstrated that the termite Reticulitermus flavipes was resistant to infection. Exposure to high levels of spores did infect a limited number of insects. Amending microcolonies of R. flavipes with sporulating termite cadavers stimulated termites to process and remove the cadavers but did not result in any detectable horizontal transmission. These data suggest that this insect possesses a strong resistance to this mycopathogen. Naïve termites were resistant to an exposure of 10⁶ conidia/g of soil. Amending this assay with sublethal concentrations of selected neoniciatoids caused termites to succumb to mycoses. A series of experiments determined that exposure to neo- nicatinoid did not act to immunosuppress the internal defenses of R. flavipes. Rather, exposure to sublethal dosages disrupted grooming behavior and tunnel formation. Normal grooming behavior results in the removal of cuticle associated conidiospores and tunneling resulted in the deposition of antagonistic bacteria that possess potent mycostatic activities.

Symposium. Wednesday, 8:45. Biocontrol of a ‘gaggle’ of termites vs biocontrol of a colonial organism – the history and future of the control of termites with entomogenous fungi

Andrew C. Rath Valent BioSciences Corporation, Asia-Pacific Research Office, 13 Hynds Road, Box Hill NSW 2765 Australia

Imported fire ants (IFA), Solenopsis spp., are invasive species that occurs over 320 million acres in the United States causing ~6.5 billion dollars per year in damage and impact on the American econ- omy. Several pathogens have been found attacking IFA in their homeland in South America and the USA but only few have been used in control efforts.

Inundative microbial control of fire ants has been plagued with variable results. The fungus Beauveria bassiana has been formulated as bait and applied to IFA-infested areas with limited success, despite encouraging laboratory results. Recent efforts with mycelial formulations in alginate pellets have produced high field mortality in Texas but no nest mortality at other locations. Further field testing of these fungal products is needed.

Inoculative releases of pathogens seem to be more adequate solu- tions to the fire ant problem in North America because these pests occur in extensive areas with little economic value where conservative control interventions are prohibitive. The protozoan Thelohania solenopae (Microsporida) has been successfully inoculated in many locations throughout the southeast USA, and seems to occur naturally in many other locations. Both polygyne and monogyne fire ant populations can be infected; however monogyne colonies found in polygyne-infested areas are usually free of the pathogen. T. solen- opae can be transmitted between nests by transfer of infected brood and within nests probably by secretion exchanges. Infected queens produce fewer eggs and the disease is transmitted transovarially to the brood. This microsporidium cause decreases in nest numbers in certain areas and increases sensitivity of infected ants to the chemical hydramethylnon used in fire ant baits.

The protozoan Vairimorpha invictae has significant impact in fire ant populations in South America but efforts to inoculate uninfected colonies have not yet been successful. The Yellow-Head Disease (YHD) caused by a Matthesia protozoan recently described from fire ants in Florida seems to have little direct impact on the ant popula- tion. This and other diseases that occur sporadically in fire ant popu- lations may be important factors in maintaining the pest population under stress and therefore less tolerant of other control agents.


Ellie Groden, Frank Drummond and Shicai Yan Dept. of Biological Sciences, Univ. of Maine, Orono, Maine 04469, USA

Myrmica rubra L. is a common species of ant in damp pastures, riverbanks and woodland edges in Europe and Central Asia. Although

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Vespula rubra is not commonly considered pestiferous in Europe. This polygynous red ant has become a severe pest along the coast of Maine where it has become locally dense and aggressively stings humans, pets, and livestock. In some areas of introduction M. rubra appears to be following the typical dynamics of an outbreak invasive species (i.e. introduction, establishment, period of adaptation, then exponential increase and geographic spread.) Many of the infested areas of the state are in close proximity to the coast and are considered environmentally sensitive. In particular, this ant has become a severe problem in many areas of Acadia National Park (ANP) on Mount Desert Island in Maine. It appears that M. rubra is having a negative impact on native species of arthropods in ANP, particularly other Formicidae. Because many infested areas include valuable natural areas and areas heavily frequented by people, there has been an expressed priority for developing a “least toxic” strategy for managing this pest that minimizes impacts on non-target organisms.

In 2002 we began collecting cadavers from middens associated with Vespula rubra on Mt. Desert Island, ME. Cadavers were surface sterilized and held at 100% RH to encourage sporulation of fungi. Isolates of Beauveria bassiana and Metarhizium anisopliae were recovered from 5.3 to 25% of the cadavers collected at each site. One isolate of each pathogen species from each site has been assayed against M. rubra workers to confirm pathogenicity, and virulence of pathogenic strains have been compared in quantitative assays. Opportunities for enhancing infection of nests are being explored. 

Symposium. Wednesday, 9:35.

New pathogens and novel strategies for Vespula control

Travis R. Glante, Andrew F. Reeson and Andrew D. Austin

The genus Vespula (Vespidae; Hymenoptera) contains a number of social predatory wasp species. In their home range they are rarely considered more than an occasional human nuisance, due to their nasty stings. However, in some areas where they have been introduced, such as New Zealand and Australia, they have become major pests. In New Zealand populations can reach more than 50,000 workers per ha and have detrimental impacts on native fauna, agriculture and people. Effective toxins are becoming available for localised control but widespread population reduction of Vespula is problematic, as many nests are in wilderness areas, or well-concealed. Virulent, self-transmitting pathogens would be a useful tool for Vespula control. Individually, Vespula are susceptible to a number of generalist pathogen species as fungi (e.g., Beauveria, Metarhizium and Aspergillus), entomopathogenic nematodes (Steinerenima and Heterorhabditis spp.) and some protozoa and bacteria. However, no nest failure has ever been attributed to pathogens. This is largely due to the well developed hygienic behaviour of social Vespula, where diseased individuals are quickly ejected from the nest. Any control strategies involving pathogens need to include methods to overcome hygienic behaviour. One approach is the use of natural microbes associated with wasp larvae and adults which are undetected by wasp workers. These could be pathogens, or microbes with the potential to be engineered to express toxins. We have examined the gut flora of Vespula wasps, looking for pathogens, commensals, symbionts and endosymbionts, through both culturing and molecular (DGGE) analysis of total populations. This has led to the discovery of several rickettsia in Vespula in Australia and New Zealand which have, potentially, some promise. The rickettsia may be a true pathogen of Vespula, or may be a target for genetic engineering. Another strategy for wasp control with pathogens is the use of behaviour disruption of the hygienic behaviour to allow nest populations to collapse through pathogens.

Symposium. Wednesday, 8:00.

Transgenic Vip Crops for Insect Control

Mikyoung Lee, Fred Walters, Hope Hart, Shank Palekar, and Eric Chen

Syngenta Biotechnology, Inc., 3054 Cornwallis Rd, Research Triangle Park, NC 27709, USA

Vegetative insecticidal proteins (Vips), which are produced during the vegetative growth stage of Bacillus thuringiensis, have been demonstrated as good source proteins for insect control applications. Among the Vips,Vip3 proteins show broad-spectrum activity against major Lepidoptera insect pests including black cutworm (Agrotis ipsilon), fall armyworm (Spodoptera frugiperda), beet armyworm (S. exigua), tobacco budworm (Heliothis virescens), corn earworm (Helicoverpa zeae), and European corn borer (Ostrinia nubilalis). Vip3 genes share no significant sequence similarity to any known genes. These genes have been transformed into both monocot and dicot crop plants and have shown potent insect control properties, being efficacious in both greenhouse and field settings. Previous histological studies (Yu et al., 1997) have shown that the Vip3A protein targets midgut epithelium cells of susceptible insects and initiates a series of cytological changes comprising profuse vacuolization and swelling of the cell walls and lysis and death. We have recently characterized the mode of action of Vip3A protein further and found that Vip3A requires an activation step by the insect gut enzymes to trigger receptor binding. BBMV ligand blotting and competition binding assays have shown that Vip3A does not share the common binding sites with Cry1Ab and Cry1Ac. Additional binding assays with two known Cry1A receptors indicate that Vip3 does not recognize these proteins. Characterizations of Vip3A by voltage-clamping of larval midgut and addition to synthetic planar lipid bilayer membranes has demonstrated that activated Vip3A forms ion channels that clearly differ from those of Cry1Ab protein. Due to these differences in the mode of action as compared to that of Bt d-endotoxins, Vip3 genes have been considered as excellent candidates for resistance management in transgenic crops.
The intracellular substrates of MTX have not been elucidated, yet. The toxin elicits a cytotoxic effect after transfection of mammalian cells. Numerous in vitro protein substrates can be found in eukaryotic and prokaryotic cell lysates, but their role in the cytotoxic effects of MTX remain to be clarified.

Symposium. Wednesday, 9:00.
Membrane permeabilizing activity of the 70 kDa moiety of the Mtx toxin from Bacillus sphaericus

Jean-Louis Schwartz2, Adaleta Maria Gaviria Rivera1, Léna Potvin3, Colin Berry2 and Gianfranco Mestrenna1

1Biotechnology Research Institute, Montreal, Que H4P 2R2, Canada; 2IFPR-DOM and Biocontrol Network, Univ. de Montréal, Que HSC 317, Canada; 3Cardiff School of Biosciences, Cardiff Univ., Cardiff, CF10 3US, UK; CNCR-ITC, Centro de Fisica degli Stati Aggregati, 1-38050 Povo, Italy

B. sphaericus (Bs) is a spore-forming bacterium that produces several mosquitocidal toxins: high toxicity strains express the binary toxins (Bin toxins: BinA, a 42kDa protein, and BinB, a 51-kDa protein), and low toxicity strains produce the mosquitocidal toxins (Mtx toxins). We showed recently that Bin, BinA and BinB permeabilize phospholipid vesicles under specific pH and lipid composition conditions, and form ionic pores in planar lipid bilayers (PLBs). BinA was principally responsible for pore formation in lipid membranes, with BinB, the binding component of Bin, playing a role in promoting channel activity. A 97-kDa protein, derived from the 100-kDa Mtx1 toxin by deletion of a putative signal sequence, is processed by mosquito larval gut juice and trypsin to a 27-kDa, N-terminal peptide (P27) and a 70-kDa, C-terminal peptide (P70). Mtx toxins are members of the ADP-ribosylating toxin family which includes several bacterial toxins. Cytolytic activity of Mtx requires the presence of both the P27 and P70 moieties. The former is the ADP-ribosylating peptide and the latter has been proposed to constitute the putative binding domain and participate into toxin entry into target cells. In the present study, we tested the hypothesis that the mode of action of Mtx toxin is dominated by the membrane permeabilization step induced by its P70 C-terminal fragment. To do so, we tested its ability to induce calcein leakage in liposomes and form ion channels in PLBs. Calcein release from P70-exposed liposomes depended on lipids, pH and peptide concentration. Optimal permeabilization took place at pH 4.5 in PC:PI (1:1) liposomes. The success rate of P70 incorporation in PLBs was around 25% under the most efficient experimental conditions, i.e., P70 concentration of at least 10 μg/ml, presence of negatively charged lipids and bath solution pH of 4.5. P70 channels displayed multiple current levels with at least one channel remaining open. Current flickering activity was generally observed. Channel conductances were determined by measuring the amplitude of all current levels with at least one channel remaining open. Success rates were 15% for the 14-kDa component (15-300 pS), 18% for the 44-kDa component (25-360 pS) took much longer to display channel activity. Success rates were 15% for the 14-kDa component (15-300 pS), 18% for the 44-kDa component (15-750 pS) and 32% for the 40-kDa fragment (8-430 pS). Furthermore, the full toxin and its individual components permeabilized liposomes. The overall membrane permeabilization process of PS149B1 may result from both pore formation and phospholipid bilayer disruption.

Symposium. Wednesday, 2:00.
Photorhabdus and Xenorhabdus genes for use in transgenic plants


Dow AgroSciences, 9330 Zionsville Rd., Indianapolis IN 46268, USA

Nematophilic bacteria from the genera Photorhabdus and Xenorhabdus produce orally active, insecticidal proteins active against a wide range of arthropod pests including economically important Coleoptera, Lepidoptera, Diptera, and Acarina. These proteins, and their corresponding genes, are distinct from the Bacillus thuringiensis proteins and genes that are currently deployed in transgenic plants. Transgenic plants containing genes from these bacteria represent exciting new tools for insect pest management. Several genes from each of three distinct “toxin complex” classes have been cloned and co-expressed in bacterial systems. Depending on the combination of genes expressed, insecticidal potency and spectrum vary widely.

Symposium. Wednesday, 2:30.
Toxins from Xenorhabdus species

Alun Morgan, Martin Sergeant, Margaret Ousley, Laura Baxter, Debbie Ellis, Heidi Sirs, Sarah Lee and Paul Jarrett

Horticulture Research International, Wellesbourne, Warwick CV35 9EF, UK

Toxins from Xenorhabdus species offer alternatives to complement existing Bacillus thuringiensis insecticides. Strains of Xenorhabdus have been identified that kill a range of insect pests including Pieris basicae, Pieris rapae, Plutella xylostella, Heliothis virescens, H. zea, and Phaedon cockleariæ; and specific strains also kill the nematode Caenorhabditis elegans. We have identified and expressed a series of insecticidal toxin genes from X. nematophilus and X. bovieni strains. The toxin genes reside on mobile elements located either on classical pathogenicity islands, or on a transposon on the chromosome of strains. Three proteins (xptA, xptB and xptC -like) are required to kill insects and insect cell lines. The XptA – like toxins control (in part) the insect host range, while the other two toxins are also required to kill the insect. The interaction of the three toxins was found with both Photorhabdus and E. coli expressed genes. Proteolytic processing to activate the toxins does not appear to be required, and results from light-scattering assays also indicate that the toxins do not act through pore formation. The nematocidal activity identified in one strain has been characterised and requires two proteins, xpt1 and xpt2. These act quickly to kill the model nematode C. elegans, and their mode of action is being investigated using traditional microscopic methods, and microarray technology investigating changes in host cell gene expression. The results from this work are encouraging and these proteins offer potential alternatives to Bacillus thuringiensis toxins

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for use in pest control, as well as providing information on pathogenicity factors associated with the ‘insect pathogen’ Xenorhabdus.

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

### VIRUSES – 4

**Contributed paper. Wednesday, 8:00.**

**Use of dsRNA to generate transgenic silkworms resistant to BmNPV**  
Ryoko Isohe, Takahiro Matsuyama, Katura Kojima, Toshihiko Kanda, Ken Sahara, Shin-ichiro Asano and Hisanori Bando

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Recently we have demonstrated that the dsRNA is a powerful tool for gene-specific gene silencing in a silkworm ovarian cell line, BmN. In this study, we examined use of the gene silencing technique in generating transgenic silkworms resistant to the Bombyx mori nucleopolyhedrovirus (BmNPV). A transient expression experiment using BmN cells demonstrated that the expression of dsRNA possessing the sequence of lef-1 which is essential for viral DNA replication, strongly suppressed the replication of BmNPV. Using a transposon piggyBac system, we generated the transgenic BmN cells (rBmN-lef1) carrying the artificial gene designed for expressing lef-1 dsRNAs. An NPV DNA microarray analysis revealed that the accumulation of lef-1 mRNA was successfully inhibited in rBmN-lef1 infected with BmNPV. And a marked reduction in the production of BmNPV was observed, i.e., the virus titers in the cultured medium of rBmN-lef1 at 48 h post infection was about 50% of control BmN cells. The BmNPV-resistance caused by the transgenesis of the dsRNA-expressing gene was analyzed in the transgenic silkworms. Fourth-instar (first day) larvae of G2 transgenic or control silkworms were orally inoculated with BmNPV polyhedra, transferred to an artificial diet and reared individually. At 72 h and 96 h after inoculation, the viral DNA in the hemolymph was quantitated by the real-time PCR. The increasing of virus in hemolymph of transgenic silkworms was suppressed at least for 96 h after inoculation of polyhedra.

**Contributed paper. Wednesday, 8:15.**

**Identification and characterization of the inhibitor of apoptosis gene of the entomopoxvirus from Amsacta moorei (AmEPV)**  
Qianjun Li and Richard Moyer

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One role of baculovirus encoded p35 and the inhibitor of apoptosis (iap) genes is to extend the life of the infected host cells thus allowing a productive virus replication. Unlike the vertebrate poxviruses, Amsacta moorei entomopoxvirus (AmEPV) encodes an iap gene. The ability of AmEPV iap to inhibit apoptosis was assayed in an in vitro transfection system and compared with known anti-apoptotic genes, including the AcMNPV p35 and Op-iap3 genes, all cloned into the pCI-E1-4 expression vector. The AmEPV iap gene can inhibit apoptosis resulting from various apoptosis inducers, including the Drosophila apoptosis gene reaper and actinomycin D. In a second assay, apoptosis leading to a non-productive infection results when S9 cells are infected with AcMNPV lacking p35 gene (AcMNPVAP35), but AcMNPVAP35 virus replication and occlusion body formation could be rescued when cells were transfected with pCI-E1-4.iap. To determine whether the iap gene is an essential gene for virus growth, we constructed an iap knockout recombinant virus in which the iap gene was replaced with b-galactosidase gene under the control of the cowpoxvirus AT1 promoter. The AmEPV(Sph+20)::GFP was used as parental virus which expresses GFP under the control of late spheroidin promoter. Although we observed a 10-fold lower virus yield for the iap knockout virus in Ld652 cells compared to the parental virus, the iap knockout virus can be propagated in Ld652 cells with normal plaque formation, indicating the iap gene is non-essential under these conditions. Further analysis showed that neither virus formed plaques in non-permissive SL2 cells, but the ability of the iap knockout virus to form plaques was significantly impaired compared to parental virus in semi-permissive S9 cells. Caspase-3 activity, an indicator of apoptosis, was also significantly increased in Ld652, S9 and SL2 cells infected by the iap knockout virus compared to cells infected by the parental virus. The above results demonstrate that the AmEPV iap gene is functional and active during AmEPV infection, and that it may act to prevent apoptosis.

**Contributed paper. Wednesday, 8:30.**

**Pariaucito nodavirus wild-type virus particles contain a minor protein translated from the second AUG codon of the capsid open reading frame**  
Karyn N. Johnson and L. Andrew Ball

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The *Nodaviridae* comprise a family of non-enveloped isometric RNA viruses that infect either insects (genus *alphanodavirus*) or fish (genus *betanodavirus*). The alphanodavirus *Pariaucito virus* (PaV) was isolated in Peru from the Southern armyworm, *Spodoptera eridania*. PaV can be propagated experimentally in the *Helicoverpa zea* cell line FB33 and in larvae of the wax moth, *Galleria mellonella*. PaV particles are about 30 nm in diameter with T=3 icosahedral symmetry. Virus particles are assembled from 180 copies of the 43 kDa capsid protein precursor alpha which is cleaved autocatalytically into two mature capsid proteins beta and gamma (39 kDa and 4.2 kDa respectively). Each virion encasipidates one copy of each of the messenger sense genomic RNA segments: RNA1 (3011 nt), which encodes the RNA-dependent RNA polymerase, and RNA2 (1311 nt), which encodes the capsid protein precursor. The 3Å crystal structure of PaV shows that the N-terminal regions of the 60 subunits surrounding the 5-fold axes interact extensively with highly ordered regions of the encapsidated genomic RNAs. Western blot analysis showed that purified preparations of wild-type PaV contained low amounts of a protein that was somewhat smaller than the mature 39 kDa capsid protein beta. This protein had a Mr of approximately 35 kDa and was also present in virus recovered from infectious cDNA clones of PaV RNAs 1 and 2. We used reverse genetics to test the hypothesis that the 35 kDa protein was produced by translational initiation at the second AUG codon, which encodes Met25 in the capsid protein open reading frame. When Met25 was mutated to Ile (M25I), the 35 kDa protein was no longer detected in transfected cells. In contrast, it was over-expressed in cells transfected with an RNA2 plasmid in which the AUG for Met1 was mutated to AGC. These data suggest that the 35 kDa protein lacked the N-terminal 24 residues of the capsid protein, and was made by a late translational start rather than by cleavage of the full-length capsid protein. When the proteins were expressed independently, particles were assembled from either full-length M251 capsid protein or from the late-start capsid protein. Interestingly while the late-start protein is found in all wild-type virus preparations, virus recovered with the M251 mutation was infectious both for the FB33 cell line and *G. mellonella* larvae.

**Contributed paper. Wednesday, 8:45.**

**Expression and purification of an active superoxide dismutase from Amsacta moorei entomopoxvirus (AmEPV)**  
Marie N. Becker, Alison Bawden, Danielle Arambulo, William Greenleaf, Richard Moyer

Depts. of Molecular Genetics and Microbiology and Pharmacology, Univ. of Florida, Gainesville, FL 32610

The entomopoxvirus from *Amsacta moorei* (AmEPV) can be readily propagated in a cell line derived from *Lymantria dispar*, Ld652 cells, and the genome has been completely sequenced making it an ideal tool to study host-virus interactions. One of the unique genes found in AmEPV is a superoxide dismutase (sod) encoded by ORF AMV255. Sequence homology to *sod* is found in most vertebrate poxviruses but not in the other completely sequenced entomopoxviruses. Superoxide dismutases (SOD) catalyze the conversion of superoxide radicals (O₂⁻...
to hydrogen peroxide and oxygen. There are 3 classes of SODs
categorized by their metal ion binding partner, either Fe, Mn or Cu
and Zn. Sequence analysis predicted the AmEPV SOD is of the
copper-zinc binding type with a monomeric size of 16.2kDa, however
unlike the vertebrate poxvirus SODs, which are inactive, the AmEPV
SOD has all of the key amino acid residues necessary for full
function. We have cloned the AmEPV sod gene into a pET
expression vector (Novagen) and expressed the protein in E. coli
with exogenously added Cu2+ and Zn2+. The recombinant protein contains
a 6x-His N-terminal epitope tag and the protein was purified using
Ni2+ affinity chromatography. The resulting protein was determined to contain 42% Cu, 53% Zn and 5% Ni. The recombinant vertebrate
erpoxvirus SODs, is active in both an in situ gel assay of
electrophoretically separated proteins and as determined by stopped
flow spectrophotometry. The kcat/Km is 4 x 10^7 M^-1 sec^-1 and is not
PH dependent. We have determined via Northern analysis that the sod
mRNA is produced later in infection. Furthermore we have demonstrated the in situ gel assay, that the viral SOD is active
in infected cells. Finally we have confirmed the presence of SOD in
AmEPV infected cells with a monoclonal antibody prepared against the
purified SOD. In order to assess the function of SOD in viral
growth and pathogenesis we have engineered viruses that are deleted for
a portion of the sod gene and contain an insertion of either GFP or
theDsRed2 fluorescent protein and we have shown that the SOD is not
required for virus growth. Therefore, AmEPV contains the first
example of an active superoxide dismutase encoded by a poxvirus.

Contributed paper. Wednesday, 9:00.
Functional analysis of AcMNPV exon0 (orf141)
that codes for a novel RING finger protein
Xiaojiang Dai1, Taryn Stewart2, Joseph Ajay Pathakamuri2, and David A. Theilmann2
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RING finger proteins mediate diverse cellular processes, e.g., onco-
genesis, apoptosis, development, viral infection, transcriptional
regression and ubiquitination. AcMNPV exon0 ORF is 786 bp and
contains codes for predicted 261 aa protein. This protein contains a novel
RING finger motif (C4YC) in which a tyrosine residue is present instead
of the normal histidine. This motif is conserved in all baculovirus homologs except for SpliNPV, which contains C3FC4. Previous analysis of OpMNPV exon0 has shown that exon0 is expressed as a late gene and all early transcripts from this gene region
are spliced to form ie0. The role of late gene, exon0, in the AcMNPV
life cycle has not been determined. Previous attempts to delete exon0
from the AcMNPV genome by homologous recombination in Sf9
cells were not successful suggesting that exon0 may be essential for
viral infection. In this study, we utilized AcMNPV BACmids to
generate exon0 knockout viruses (AcMNPV-exon0-KO) by
recombination in Escherichia coli. As a control, AcMNPV exon0-
repair bacmids were generated by transposition of the exon0 gene into
the polyhedrin locus of the AcMNPV-exon0-KO BACmid. These
AcMNPV BACmids are being analyzed for AcMNPV infectivity in
comparison to wild-type viruses. Initial results indicate that deletion of
exon0 severely affects the AcMNPV infection cycle.

Contributed paper. Wednesday, 9:15.
Post-translational modification of AcMNPV GP64
by palmitoylation: mapping and functional studies
genome localization
Sandy Xiaoain Zhang, Yu Han, and Gary W. Blissard
Boycy Thompson Institute at Cornell Univ., Ithaca, NY 14853, USA
The major envelope glycoprotein (GP64) of the virus AcMNPV, is
post-translationally modified by palmitoylation. By 2H-palmitate
labeling a series of C-terminal truncations and single amino acid
substitution mutations of GP64, the palmitoylation site was mapped to
a single residue at cysteine 503. We then replaced the wild-type
gene in AcMNPV with a modified gp64 gene that contained
either an alanine or serine substitution at residue 503, a mutation that
was found to abrogate palmitoylation of the GP64 protein. Using
recombinant viruses that expressed only a palmitoylation-minus form
of GP64, we examined the potential functions of GP64 palmitoylation
in the context of the infection cycle. We observed no effect of
gp64 palmitoylation on the synthesis or transport of GP64 to the cell
surface in infected Sf9 cells. We also found that the palmitoylation-
minus forms of GP64 mediated low pH-triggered membrane fusion in
a manner indistinguishable from that of wild type GP64. Thus,
palmitoylation of GP64 was not required for pH-triggered membrane
fusion by GP64. To determine if GP64 palmitoylation affected virion
production, we measured yields of infectious virions from cells
infected with palmitoylation-mutant virus. Our results indicated that
GP64 virion production was not affected as infectious virions were
generated at levels similar to those from cells infected with wild type
AcMNPV. Thus, in combination these data suggest that virus entry
into and egress from Sf9 cells were not significantly affected by
GP64 palmitoylation. We next asked whether AcMNPV GP64 was
associated with membrane microdomains known as lipid rafts, and
whether GP64 palmitoylation affected the localization of GP64 in cell
membranes. GP64 was not associated with cold detergent insoluble
membrane fractions from infected Sf9 cells although a control lipid
raft associated protein, Fasciclin I, was associated with detergent
insoluble membrane fractions. Results from these experiments show
that AcMNPV-infected Sf9 cell membranes contain lipid raft
microdomains that indicate that GP64 was not associated with lipid
rafts in infected Sf9 cells. In addition, GP64 palmitoylation did not
affect the apparent exclusion of GP64 from lipid raft microdomains.

Contributed paper. Wednesday, 9:30.
Comparative analysis of baculovirus envelope fusion protein F
and a cellular F homolog of D. melanogaster
Oliver Lung and Gary W. Blissard
Boycy Thompson Institute at Cornell Univ., Ithaca, NY 14853, USA
Baculovirus genomes have been suggested to undergo constant gene
content changes during their evolution, and exchange of genetic
material between baculoviruses and their hosts have been docu-
mented. Recent discovery of a cellular homologue of the baculovirus
envelope fusion protein F in the Drosophila melanogaster genome
suggests that baculoviruses may have acquired its budded virus
envelope fusion protein from an insect host. Consistent with this
hypothesis, F homologues have also been found in EST libraries
derived from the silkworm Bombyx mori which are normal hosts of
baculoviruses. These observations suggest that homologs of baculo-
virus envelope fusion protein gene may be present in many insects.
We examined the temporal and spatial expression as well as the
subcellular localization of the D. melanogaster cellular F homolog,
Dm-F. Using vectors that would generate Dm-F-GFP fusion protein
and Dm-F-V5 epitope tagged protein, we showed that unlike viral F
proteins which localize to the plasma membrane when transiently
expressed in cultured cells, Dm-F transiently expressed in DmS2
cells localizes to discrete cytosolic compartments within the cytosol.
In addition, our RT-PCR results suggest that Dm-F expression is
developmentally-regulated. Dm-FExpression was first detected in 3rd
instar larval, but was also detected in the pupal stages and in adults
of both sexes. Analysis of Dm-F expression and localization using
in situ-hybridization and immuno-localization will be presented.

Contributed paper. Wednesday, 9:45.
Functional analysis of the fusion domain
of baculovirus F proteins
M. Westenberg1, O. Lung2, D. Zuidema1, G.W. Blissard3 and J.M. Vlak
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2Boycy Thompson Institute, Cornell Univ., Ithaca NY 14853, USA
Envelope fusion proteins or F proteins are found in budded viruses
(BV) of group II nucleopolyhedroviruses. This group belongs to the
Genus Nucleopolyhedrovirus (Family Baculoviridae), that further
encompasses Group I NPVs. The Group II NPVs are among others
characterized by the presence of a different envelope fusion protein,
GP64. Phylogenetic analysis on the basis of a variety of genes support
this division into two groups. Envelope fusion proteins either of the F type or of the GP64 type are involved in attachment to cells, mediate membrane fusion, and are required for efficient virus budding from the cell membrane. Their function is similar to envelope fusion proteins of vertebrate viruses. The primary translation product of the F gene is the major envelope protein of BVs and is posttranslationally cleaved by a cellular proprotein convertase (e.g. furin) into two disulphide-linked subunits (F₁ and F₂). To determine whether the F protein of Group II NPVs is functionally analogous to GP64 of Group I NPVs, the gp64 gene of AcMNPV was replaced using bacmid technology by the SeMNPV F gene. Whereas transfection of the AcMNPV gp64 null bacmid into insect cells did not generate infectious particles, addition of the F gene rescued this defect. Furthermore, using a specific inhibitor it was confirmed that furin is involved in maturation of the F protein. BVs produced in the presence of the inhibitor possess the uncleaved F protein and are non-infectious. Reintroduction of an F protein with an altered furin cleavage site into the AcMNPV gp64 null bacmid rendered non-infectious virus confirming the importance of the cleavage for viral infectivity. In several animal viral fusion proteins such cleavage activation results in a conformational change releasing an alpha-helical ‘fusion domain’ with a hydrophobic side which can then interact with host cell membranes. The N-terminus of F₁ possess common features with such a fusion domain. To study the role of individual amino acids in the fusion process the effect of mutations in this putative fusion domain on viral infectivity was measured. Mutant F genes with single amino acid mutations were introduced in AcMNPV gp64 null bacmids. None of the mutations had an effect on the processing and incorporation of F proteins in the envelope of BVs, but some of these mutations had a dramatic effect on viral infectivity.

**SYMPOSIUM (Cross-Divisional), Wednesday, 2:00–4:00.**

**Epizootiological modeling**

Symposium. Wednesday, 2:00.

**Entomophaga maimaiga and the Gypsy Moth: Insights from a model**

Ronald M. Weseloh

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The fungal pathogen, *Entomophaga maimaiga*, suddenly appeared in New England in 1989 and spread at a rate of about 200 km per year until it essentially covered the entire North American gypsy moth range. It overwinters as resting spores in the soil, and under appropriate moist conditions in the spring these germinate and infect gypsy moth larvae. Infected larvae eventually die and produce airborne conidial spores that infect other larvae if humidity is high enough. The importance of the interactions between temperature-dependent growth and moisture conditions for effectiveness of the pathogen lead to the development of a simulation model of infection that incorporates temperature, humidity, resting spore load in the soil, and gypsy moth density. The model fit forest data on fungal prevalence best if conidial spores from local sites were allowed to mix freely before infecting larvae. Thus, local dispersal of spores is very important and helps explain the density independence of infection prevalence. The model was also used to explore the potential of the fungus for long distance dispersal. Simulations conducted using weather conditions during the years immediately after 1989 showed that the fungus would have been able to spread rapidly and cause noticeable infections in new areas, as was observed. A simplified version of the model that may be of some use to forest managers is described.

Symposium. Wednesday, 2:25.

**The dynamics of inoculum persistence in the infection of the Colorado potato beetle with *Beauveria bassiana***

Francis A. Drummond and Eleanor Groden

Dept. of Biological Sciences, Univ. of Maine, Orono, ME USA

A simulation model of the Colorado potato beetle life history in Maine and its interaction with the fungal pathogen *Beauveria bassiana* was constructed to assess the relative importance of primary infection and secondary (horizontal) infection in the larval stage.

We modeled single and successive applications of conidia to potato foliage to investigate the management strategies of timing and frequency of foliar applications for control of larval populations. Time to death and infection rate, as a function of the number of conidial applications, are both incorporated into the model. In addition, the effect of persistence of conidia on the foliage from weathering and levels of resulting infection was investigated in the field and then simulated in the model. Horizontal infection was modeled as a function of wandering larvae coming in contact with infective cadavers. The effects of single vs. sequential applications of conidia to the foliage on the subsequent horizontal infection rates were found to significantly affect total infection. Small increases in persistence of conidia on foliage were shown to have large effects on primary infection. Conversely, persistence of cadavers responsible for horizontal transmission has much less impact on subsequent infection.

**Symposium. Wednesday, 2:50.**

**Combining mechanistic and statistical modeling to predict epidemics in insect populations**

Greg Dwyer¹, Bret Elderd², and Marc Coram²

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Environmental biologists have traditionally viewed mathematical modeling and statistical modeling as different disciplines. Consequently, in this field, mathematical models are often over-parameterized and of little value for understanding data, while statistical significance sometimes has little to do with biological significance. Recent work by mathematical and statistical ecologists has begun to bridge the gap between statistical and mathematical models by showing that stochastic mathematical models can be used as statistical hypotheses about data. Here we apply this approach to data from epidemics in the gypsy moth, *Lymantria dispar*, focusing mostly on the nuclear polyhedrosis virus. In particular, by constructing a model that allows for both stochasticity in transmission and measurement error, we have produced a parameterized model that can successfully predict the timing and intensity of virus epidemics. Given measurements of initial gypsy moth densities and initial virus loads on egg masses, this model produces a prediction of the fraction of insects that will die of the virus, as well as a 95% confidence interval around this prediction. The model may therefore be useful for predicting the minimum disease mortality in years in which rates of infection with the fungus *Entomophaga maimaiga* are low; in ongoing work, we are extending the model to predict rates of infection with the fungus as well. In addition, by using the model in a Bayesian statistical framework, we have shown that estimates of model parameters from transmission experiments are consistent with estimates from epidemic data. This in turn suggests that it is possible to predict epidemics from experimental data, which may be valuable for assessing the efficacy of different virus strains in microbial control.

Symposium. Wednesday, 3:15.

**Modeling Nosema disease in honey bee colonies**

David W. Onstad¹, David W. Crowder¹, and Zachary Huang²

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*Nosema* disease is an important problem for beekeepers around the world. We will present the conceptual and mathematical formulation of the model. We will also compare this model to previously published models of microsporidian epizootiology.
Mapping Binding Epitopes on Cry Proteins

Mohd Amir F. Abdullah1, Autumn White1, Rebecca J. McNali1, Michael J. Adang2 and Donald H. Dean1

1Dept. of Biochemistry, The Ohio State Univ., Columbus, Ohio 43210, USA;
2Dept. of Entomology, Univ. of Georgia, Athens, Georgia, USA

A large number of studies from several laboratories have revealed binding epitopes on Cry proteins in domains II and III. We have used this information as well as molecular modeling to identify binding epitopes in Cry4Ba and Cry19Aa. Site-directed mutagenesis was employed on Cry4Ba to enhance activity against Anopheles and create Culex activity (which is not detectable in wild type Cry4Ba). Similarly Aedes activity was introduced into Cry19Aa which otherwise is very low. The level of activity of modified Cry4Ba against Cry pips was 70 ng/ml, representing greater than a 700-fold increase in toxicity. Anopheles activity was enhanced 10-fold in Cry4Ba to a new level of 3 ng/ml. Toxicity of modified Cry19Aa was enhanced to a level of 3.3 ng/ml representing a 42,000-fold increase.

Symposium. Wednesday, 3:30.

Receptors and rafts in Cry toxin action

Meibao Zhuang1,2, Ruiyu Xie1,2, Isabel Gomez3, Mario Soberón3, Alejandro Bravo4, Linda S. Ross2 and Sarjeet S. Gill1

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Bacillus thuringiensis Cry protein exerts its toxic effect through a receptor-mediated process. Both aminopeptidases and cadherin-like proteins were identified as putative Cry1A receptors from Heliothis virescens and Manduca sexta. The importance of cadherin-like protein was implied by its correlation with a Cry1A resistant H. virescens strain (Gahan et al., 2002, Science), while suppression of aminopeptidase-N (APN) and cadherin-like proteins (Bt-R1) are the putative receptors for Cry1A toxins. The binding affinity of APN is on the range of 100 nM while that of Bt-R1 is on the range of 10 nM. The differences in binding affinities between APN and Bt-R1 suggest that binding to Bt-R1 is the first event on the interaction of Cry1A toxins with microvilli membranes and, therefore, the primary determinant of insect specificity. However, the precise role of the two receptors in the mode of action of Cry toxins still remains to be determined. Our results show that specificity of Cry1A involves at least two structural determinants on the Bt-R1 receptor. Incubation of Cry1Ab protoxin with two Bt-R1 peptides of 70 residues, corresponding to the two toxin binding regions, or with a single chain antibody scFv73 that mimics Bt-R1 receptor, and treatment with M. sexta midgut juice, resulted in the formation of a 250 kDa oligomer composed of four Cry1Ab toxin monomers that lacks the helix a-1 of domain I. Cry1Ab protoxin was also activated to a 250 kDa oligomer by incubation with brush border membrane vesicles by the action of a membrane associated protease. We will present the comparison of the membrane insertion capabilities of the 250 kDa pre-pore with that of the monomer. These data shows that cadherin receptor binding allows the efficient cleavage of a-1 and formation of a pre-pore oligomeric structure that is efficient in pore formation. Finally we will present data showing that a pre-pore structure is also formed on other Cry toxins after proteolytic activation in the presence of their receptors, suggesting that the pre-pore formation is a general membrane insertion intermediate of 3-domain Cry toxins.

Symposium. Wednesday, 2:00.

Bacillus thuringiensis Cry1 toxin activity: role of domain I components and modulation by the physico-chemical environment

Vincent Vachon1,2, Raynald Laprade1,2, Jean-Louis Schwartz1,2,3 and Luke Masson1,2

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The mechanism by which Bacillus thuringiensis insecticidal toxins form pores in the luminal membrane of midgut epithelial cells of susceptible insects remains one of the most challenging questions regarding their mode of action. Following solubilization in the midgut lumen and binding to specific receptors on the surface of the intestinal membrane, the toxins insert into the membrane and form pores. The process of membrane integration and permeabilization is believed to involve extensive conformational changes in the toxin molecule and assembly of an oligomeric structure composed of a yet undetermined number of toxin subunits. In three-domain toxins, domain I amphipathic a-helices are thought to play a crucial role. In this presentation, our recent work on the role of different domain I components of Cry1Aa will be summarized with emphasis on the analysis of the functional properties of an extensive collection of mutants using a variety of biophysical techniques. Strong evidence for conformational changes involving displacements of domain I helices away from each other was first obtained with engineered disulfide bridge mutants. Consistent with the fact that such a reorganization must involve movements about the polypeptide backbone in interhelical loops, several mutants with alterations in domain I loop residues displayed significantly reduced rates of pore formation. Helix 4 was shown to line the lumen of the pore using in situ site-directed chemical modification. Comparison of a large number of mutants with single-site substitutions in helices 3 and 4 stressed further the importance of helix 4 in the mechanism of pore formation. The efficiency with which toxins form pores also depended on several
factors which are likely to influence the surface properties of the membrane. Among them, pH, ionic strength, midgut proteases and possibly membrane potential strongly alter the functional properties of several toxins. Interestingly, the influence of these factors can differ greatly depending on the toxin being studied, even for closely related toxins such as Cry1Ac and Cry1C. Further elucidation of the mode of action of *B. thuringiensis* toxins will clearly require a better understanding of the contribution of these and other structural and environmental factors.

Symposium. Wednesday, 2:00.

**Functional properties of *Bacillus thuringiensis* toxin receptors in a *Drosophila* S2 cell system**

**Michael J. Adamu**$^{1,2}$, Juan L. Jurat-Fuentes$^1$, and Gang Hua$^1$

Depts. of *Entomology and Biochemistry and Molecular Biology*, Univ. of Georgia, Athens, GA 30602, USA

*Bacillus thuringiensis* (Bt) toxin mode-of-action research aims to elucidate how toxin, receptor and cell components interact leading to cytotoxicity. Due to the complexity of toxin action, no single *in vitro* assay adequately measures all aspects of toxin function. For example, brush border membrane vesicles have been invaluable in toxin binding assays, pore formation assays and as a source of toxin binding proteins. Cultured cell assays have provided insights into channel formation properties of toxin. A challenge is to compare results obtained using different *in vitro* assays. This has become more important as cDNAs encoding candidate Bt receptors have been expressed in cultured cells. Establishing function as receptors has been challenging for both classes of candidate receptors: amino-peptidases and cadherin-like proteins. This talk will address our approach to developing a functional cell-based assay for Cry toxin receptors. We selected *Drosophila* S2 cells for this purpose as they are not killed by Cry1 toxins, readily transfected with plasmid DNA and non-lytic protein expression plasmids are available. *Drosophila* S2 cells were transfected with a dual promoter plasmid that expressed a Green Fluorescent Protein (GFP)–zeocin fusion protein and the candidate receptor to be tested. For our purposes GFP functioned as a fluorescent indicator of transfectant properties. Qualitative toxin binding was visualized with fluorescently tagged Cry toxin and quantitative binding determined using $^{125}$I-labeled toxin. The fluorochrome propidium iodide (PI) served as a cytotoxicity marker. Using either confocal or inverted fluorescent microscopy, cells were inspected for GFP–fluorescence, bound toxin and cytotoxicity. Cells were also scored for these fluorescent events by flow cytometry. The results were not always predicted from other types of assays. For example, Cry1Aa binds 120-kDa APN from *Bombyx mori* and is cytotoxic to cells expressing APN. However, while Cry1Ac binds cells expressing 110-kDa APN from *Heliothis virescens*, those cells are not killed by toxin (Banks et al. 2003). Results with expressed Br-R1 cadherin were less surprising as Cry1Aa toxin binding was positively correlated with S2 cytotoxicity. Truncated forms of Br-R1 were also expressed in S2 cells and results will be discussed. Overall, S2 cells expressed APNs and cadherin in a form that bound Cry1 toxin. GFP–fluorescence proved to be an effective indicator of transfectant cells, and PI established cytotoxicity. This system holds promise for future investigations of Cry toxin action.
HaNPV/gfpΔp74 was analyzed by the flow cytometer: 34.7% normal host cells were infected by HaNPV/ gfpΔp74, while the infection ratio is 55.7% in colchicines treated cells and 7.34% in CD treated ones. The results observed under confocal immunofluorescence microscopy also showed that HaNPV nucleocapsid could induce the aggregation of Actin in the cytoplasm (1hr p.i.) and that the nucleocapsid could enter the nucleus (4hr p.i.). All the results indicated that HaNPV VP39 could interact with host Actin and such interaction led the nucleocapsid to transporting from the cytoplasm to the nucleus.

Contributed paper. Wednesday, 2:30.

Polydnavirus integration in gypsy moth cells
D.E. Gunderson-Rindal and D. E. Lynn
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The long-term persistence of polydnavirus (PDV) DNA in infected lepidopteran cell cultures suggests that at least some of the virus genome becomes integrated permanently into the lepidopteran cell genome. To investigate this, cloned libraries were prepared from two different Lymantria dispar (gypsy moth) cell lines that had been maintained in continuous culture for more than five years post infection with the baculovirus Gliptapanteles indiensis PDV (GiPDV). Junction clones containing both insect chromosomal and polydnarviral sequences were isolated. Precise integration junction sites were identified by sequence comparison of linear (integrated) and circular forms of the GiPDV genome segment F, from which viral sequences originated. Host chromosomal sequences at the site of integration varied between the two L. dispar cell lines though virus sequence junctions were identical and contained a palindromic repeat. The chromosomal site of one junction clone contained sequences with structural similarity to a retrotransposorn, encoding a putative reverse transcriptase and integrase, upstream of the putative site of viral integration. The GiPDV genome segment F does not encode any self-replication or -insertion proteins, suggesting a host-derived mechanism may be responsible for its in vivo integration.

Contributed paper. Wednesday, 2:45.

A phage-displayed peptide can inhibit infection of white spot syndrome virus of shrimp
Guohua Yi, Yipeng Qi, Juan Qian, and Zhimin Wang
College of Life Sci., Wuhan Univ., Wuhan, Hubei, 430072, P.R.China

Although white spot disease (WSD) caused by white spot syndrome virus (WSSV) results in devastating losses to shrimp farming industry around the world, no effective treatments have been found. Control focuses on exclusion of the virus from culture ponds but once introduced, spread is often rapid and uncontrollable. The purpose of this study was to select a phage-displayed peptide that might be able to prevent WSSV infections. Thus, a 10mer phage display peptide library (titer 7.2 x 10^7) was constructed and screened against immobilized WSSV. After three round of selection, 4 peptides were selected by ELISA and affinity constants determination. The 4 selected peptides were further assessed for specificity and inhibition efficiency for viral infection. The affinity constants determination and the plaque reduction neutralization test (PRNT) in primary cell cultures indicated that of 4 peptides that specifically bound with WSSV, one designated 2E6 had high specificity (affinity constant Kd is 7.28 x 10^-11) and appeared capable of inhibiting virus infection completely at a peptide concentration of about 400 mmol per ml. A similar result was seen in the whole animal tests. That is, peptide 2E6 gave the lowest mortality (33.38%) and the longest LT50 (more than 20 days). The sequencing results showed the possible critical motif for viral inhibition being YAVNNNSY. Altogether, our results suggested that peptide 2E6 had potential for exploitation as an antiviral peptide drug.

Contributed paper. Wednesday, 3:00.

Providence virus: a new tetravirus with an unusual arrangement of its non-structural genes
Fiona M. Pringle, Karyn N. Johnson and L. Andrew Ball
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Tetraviruses are small, positive-sense RNA viruses that specifically infect Lepidopteran insects. The two genera within the Tetravirusidae are distinguished on the basis of their monopartite or bipartite genome organization, capsid morphology, and capsid protein sequence homology. Providence virus (PrV) is a novel tetravirus that was discovered as a persistent infection of a Helicoverpa zeae midgut cell line. The capsid morphology, monopartite genome organization, and capsid precursor protein processing of PrV are similar to viruses from the betatetravirus genus. However, the PrV capsid protein sequence was more similar to those of omagaretavirus. In addition, the genome arrangement of PrV differs from that of other tetraviruses. The 6.2 kb PrV genomic RNA encodes three open reading frames (ORFs). The 5'-proximal ORF encodes a 140 kDa protein of unknown function and overlaps a second non-structural ORF for 2683 nt (98% of ORF2). ORF2 encodes the putative RNA-dependent RNA polymerase (RdRP), but it is interrupted by a stop codon. The ORF2 protein sequence has little similarity to other tetravirus RdRPs, but resembles RdRPs from the Tombusviridae, a family of plant positive-sense RNA viruses. Tombusvirus RdRP ORFs are also interrupted by a stop codon, read-through of which yields two non-structural proteins. The 3'-proximal PrV ORF encodes the capsid protein precursor, which is processed twice to yield the two capsid proteins and a small non-structural protein of unknown function. A similar mechanism has also been described for Thosia asigna virus (TaV) and Euprosterna elaeca virus (EeV). In all three viruses, an 18 amino acid sequence at the C-terminus of the small non-structural protein is similar to the self-cleaving sequence described for the Foot and mouth disease virus 2A protein. PrV encodes two additional 2A-like sequences, both of which are within non-structural proteins. If these 2A-like processing sites are active and two proteins are produced from the RdRP ORF, PrV could produce up to nine proteins from its genome. The characteristics of PrV, together with the recently described permuted RdRPs of TaV and EeV, are significant reasons for reassessing the taxonomy of the Tetravirusidae.

Contributed paper. Wednesday, 3:15.

Baculovirus diversity: Establishment of a natural classification system using molecular phylogeny
Martin Lange, Hualin Wang and Johannes A. Jehle
State Education and Research Center for Agriculture Viticulture and Horticulture (SLFA), Biotechnological Crop Protection, Breitenweg 71, 67435 Neustadt/Wstr., Germany

Baculoviruses form a large and diverse group of DNA viruses, which are pathogenic for invertebrates. These viruses have been predominantly isolated from members of the Insecta and were successfully used as natural insecticides against insect pests. In respect to their high host specificity and large diversity there is a growing interest for a fast and reliable method to identify and classify newly isolated baculoviruses. We have developed a PCR-based method for the detection and taxonomic identification of lepidopteran specific baculoviruses using hierarchical degenerated primer pairs. Viral DNAs from 50 infected lepidopteran species were isolated using a commercial kit (Qiagen). Highly conserved DNA sequences within the coding regions of three baculovirus core genes (polyhedrin, le-5, le-9), one GV specific gene (CpGV ORF22 homologues) and one NPV group I specific gene (gp64) were targeted for PCR amplification. Database searches and phylogenetic analyses of cloned and sequenced PCR products from these conserved genes revealed that many of the sequences can not be assigned to classified baculoviruses and likely belong to new taxa.
Baculoviruses are large (~100–180 Kbp) double stranded DNA viruses that infect lepidopteran, hymenopteran and dipteran insects. Many of the host insects are significant agricultural pests and baculoviruses have been used as biocontrol agents. As such, there has been considerable research into their molecular biology, population genetics and epidemiology. To date, 18 fully sequenced baculovirus genomes from 16 different host species have been determined. BUGs is a generic bioinformatics pipeline that undertakes comparative genomics and outputs the data in tabular or graphical form for rapid interrogation by biologists. The pipeline is largely dependent on Blast searches of locally held baculovirus proteomes and genomes to identify shared genes/proteins and sequences. Homologues identified by Blast searches are then automatically sorted to reveal gene distributions (presence, absence and paralogues) among the genomes, gene order comparisons (synteny) the phylogenetic relationships of each protein and the identification of pseudogenes. The implications that the output data has for baculovirus evolution will be presented.

Symposium. Wednesday, 4:30-6:30.

Baculovirus genomics

Symposium. Tuesday, 4:30.

Genomics and evolution of the Neodiprion lecontei “nucleopolyhedrovirus”

Basil Arif1, Hilary A.M. Lauzon1,
Christopher Lucarotti2 and Peter Krell3

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Hymenoptera represents a more ancient order of insects than Lepidoptera and while the total sequences of a number of baculoviruses infecting the latter have been reported, only recently, genomic sequences of NPVs from hymenoptera have been determined. The genome of the nucleopolyhedrovirus infecting the redheaded pine sawfly, Neodiprion lecontei (NeleNPV) is the smallest sequenced so far measuring 81,756 base pairs. It has a high A+T contents residue (67%) indicating a bias of codon usage. Most of the genes associated with transcription, DNA replication and those encoding viral replicators, even without prior knowledge of the mechanisms governing host range and virulence. To identify baculovirus genes that have undergone positive selection, a maximum likelihood approach was used to analyze selection pressures acting on genes of nucleopolyhedroviruses (NPVs) that have different host ranges within the Lepidoptera. Alignments of eighty-six group 1 NPV genes were fitted to models of codon substitution that allow for varying selection intensity among codon sites. Evidence for positive selection was found for thirteen genes: ac38, ac73, ac103, dnahel, dnapoi, lef-10, lef-12, odv-e16, odv-e56, p6.9, pki, vp39, and vp80. NPV genes that have undergone positive selection may modulate the ability of different NPVs to replicate efficiently in cells (dnahel, dnapoi, lef-10, lef-12) or establish primary infection of the midgut (odv-e18, odv-e56) of different host species.

Symposium. Tuesday, 5:42.

Influence of hosts on the diversity of the Baculoviridae

Elisabeth A. Herniou1,2, Julie Olzewski1,
David O’Reilly3 and Jenny Cory2

1Dept of Biological Sciences, Imperial College London, London, UK; 2NERC CEH-Oxford, Oxford UK – 3 Syngenta, Bracknell, UK

Baculoviruses are primarily insect pathogens, infecting mostly larvae of the Order Lepidoptera but also some Diptera and Hymenoptera. The diversity of the Baculoviridae was investigated with an emphasis on how it relates to the phylogeny of host species. Sequences of the polyhedrin, lef-8 and ac22 genes were acquired from historical field isolates of infected insects. These were employed to reconstruct molecular phylogenies to describe baculovirus evolutionary relationships. The trees were first used to investigate the relationship between virus isolates and virus species. Then baculovirus species have undergone adaptive evolution, i.e., positive selection. Given that the primary selection pressure on a virus results from virus-host interaction, positively selected virus genes may facilitate infection and replication. Analysis of selection pressure on virus genes can potentially identify genes involved in virulence or in crossing species barriers, even without prior knowledge of the mechanisms governing host range and virulence. To identify baculovirus genes that have undergone positive selection, a maximum likelihood approach was used to analyze selection pressures acting on genes of nucleopolyhedroviruses (NPVs) that have different host ranges within the Lepidoptera. Alignments of eighty-six group 1 NPV genes were fitted to models of codon substitution that allow for varying selection intensity among codon sites. Evidence for positive selection was found for thirteen genes: ac38, ac73, ac103, dnahel, dnapoi, lef-10, lef-12, odv-e16, odv-e56, p6.9, pki, vp39, and vp80. NPV genes that have undergone positive selection may modulate the ability of different NPVs to replicate efficiently in cells (dnahel, dnapoi, lef-10, lef-12) or establish primary infection of the midgut (odv-e18, odv-e56) of different host species.
trees were used to reconstruct the ancestral host use of the lepidopteran baculoviruses. The results indicate that the host of the ancestral lepidopteran baculovirus was likely to have belonged to the family Noctuidae. Furthermore, the phylogenetic analyses showed that the evolution of baculovirus host affiliation is characterised by phylogenetic conservatism. This is reflected both at the family level within the Lepidoptera, and at the insect order level. A separate phylogenetic analysis showed that baculoviruses of hosts from the Lepidoptera, Hymenoptera and Diptera cluster separately. Thus, surveying the diversity of the Baculoviridae clearly indicated an evolutionary link between baculoviruses and their hosts.

Symposium. Tuesday, 6.06.

Complete genome comparison of two baculoviruses that are highly pathogenic for the cabbage looper; *Trichoplusia ni* single nucleopolyhedrovirus (Group II NPV) and *Autographa californica* nucleopolyhedrovirus (Group I NPV)

Leslie G. Willis1, Taryn Stewart2, Robyn Seipp3, Martin Erlandsen4, and David A. Theilmann1

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The cabbage looper *Trichoplusia ni* is becoming a serious pest of the greenhouse industry in the Fraser Valley of British Columbia. Two naturally occurring baculoviruses have been identified that infect and kill *T. ni*. They are *T. ni* single nucleopolyhedrovirus (TnSNPV) and *Autographa californica* multiple NPV (AcMNPV). These two viruses belong to two distinct evolutionary lineages and have different biological properties. TnSNPV is a Group II NPV and appears to have a narrow host range, whereas AcMNPV, a Group I NPV, has a very broad host range, but both viruses are highly virulent for *T. ni* early instar larvae. To determine the molecular basis for the biological differences between these viruses the complete genome of TnSNPV has been sequenced and compared to the AcMNPV genome. The TnSNPV genome was found to be 134,395 bp and code for over 130 genes with open reading frames of 150 nucleotides or longer. Comparative analysis of these two viruses has shown they contain many genes that are evolutionarily related, as well as unique genes that may play a role in the observed biological differences.

**FUNGI – 3**

**Contributed papers. Wednesday, 4:30-6:30.**

*Phylogeny of the insect pathogenic fungus, *Metarhizium*

Michael J. Bidochka and Cherrie L. Small

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We have re-assessed the data from several studies on the population genetics of *Metarhizium* in an attempt to place a phylogeographic perspective on the world-wide population structure of *Metarhizium*. Clearly, some direction is needed in order to coalesce the data on the population genetics of *Metarhizium*. Here we forward the following hypotheses based on supportive data from several publications, as well as data from our laboratory, on the phylogeography of *Metarhizium*. These are: (1) South-East Asia is probably the origin in the evolution and diversity of *Metarhizium*, (2) similar genotypes of *Metarhizium* can traverse continental distances, (3) associations of *Metarhizium* genotypes with certain host insect species probably occurs only in tropical and subtropical regions, (4) association of *Metarhizium* genotypes occurs with habitat type in temperate and polar regions and (5) *Metarhizium* is actually an assemblage of cryptic species found world-wide. Discussion of these hypotheses should provide an evaluation of the taxonomy and species concept within the genus *Metarhizium*.

**Molecular mechanisms of adaptive radiation in *Metarhizium anisopliae***

Raymond St. Leger and Gang Hu

Dept. of Entomology, Plant Sciences Bldg., Univ. of Maryland, College Park, 20742, USA

Pathogen biodiversity is an under-exploited source of inference regarding disease processes and the evolution of pathogens and pathogenesis. However, the entomopathogenic fungus *Metarhizium anisopliae* provides an excellent model system for applying this approach. It is a radiating species, and contains both generalist and specialized lineages with broad and narrow host ranges. Strains can be selected to represent evolutionary distances ranging from <1 to 8 MY and their natural molecular variation allows analysis of processes of both adaptive change and phylectic differentiation still in operation, even in intermediate stages. We are using strains with broad or narrow host ranges, isogenic genotypes disrupted in key regulators of transcription, and thousands of cDNAs in microarray surveys to investigate: 1) the number, nature and networking of genes that regulate and execute infection processes, 2) factors controlling aggressiveness and the evolution of specificity, and 3) identify key targets for precision alterations of pathogen performance. In particular, we have assembled atlases of gene expression in strains to determine the extent to which differences in strain phenotypes derive either from changes in gene content or from shared genes having dissimilar expression patterns. Dissection of regulatory mechanisms in multiple strains has started with surveys of transcription factor binding sites from genes that are similarly or differently regulated to identify common and contrasting regulatory elements.

**A multigene phylogeny of *Beauveria*: new insights into species diversity, biogeography, host affiliation and life history**

Stephen A. Rehner

USDA-ARS, Insect Biocontrol Lab., Beltsville, Maryland 20705, USA

*Beauveria* is a genus of haploid, soil-inhabiting, entomopathogenic hyphomycetes of wide interest for their potential use as biological control agents against pest insects. Although its cosmopolitan distribution is suggestive that *Beauveria* may be cryptically diverse, traditional morpho-taxonomic assessments admit only a few species. A multilocus nucleotide data set has been acquired with which phylogenetic relationships have been reconstructed within a global sampling of strains representing *B. bassiana*, *B. brongniartii*, *B. amorpha*, *B. cedellonica* and *B. vermiconia*. Phylogenetic reconstructions of these genes reveal a congruent phylogenetic structure that resolves five well-supported clades, A-E. The morphological species *B. bassiana* is divided into two clades, A and C, and thus is polyphyletic. Clade A is globally distributed and contains the strain proposed as neotype for *B. bassiana*. Clade C contains strains from Europe and North America. Strains in both clades A and C infect a wide range of insect taxa, reinforcing traditional views that *B. bassiana* is a generalist entomopathogen. Clade B, which corresponds to *B. brongniartii*, is monophyletic and has a Eurasian distribution. Clade D includes *B. cedellonica* and *B. vermiconia*, and along with several unidentified strains, forms a disjunct complex of divergent species. Clade E, which contains strains of *B. amorpha*, forms the most basal lineage within *Beauveria* Cordyceps bassiana, *C. scarabaeicola* and *C. staphylinidae* are shown to be derived from within clades A (*B. bassiana*) and D (*Beauveria sp.*) thus indicating that, despite their mitotic mode of reproduction, sexuality is present throughout *Beauveria*. The multi-gene phylogeny of *Beauveria* provides a robust framework for comparative biological studies and their coevolutionary interaction with insects.
Phylogenetic and population genetic approaches to the analysis of cryptic speciation in the **Beauveria bassiana** s.str. complex

**Stephen A. Rehner**

USDA-ARS, Insect Biocontrol Lab., Beltsville, Maryland 20705, USA

**Beauveria bassiana** s.l. is one of the most common fungal entomopathogens encountered in nature, occurring globally and infecting a vast array of species from at least seven insect orders. To determine whether evolutionary diversification in *B. bassiana* is associated principally with geographic or host origins, a multi-gene phylogeny based on partial sequences of eight nuclear genes was constructed for a global sampling of *B. bassiana* strains. Using phylogenetic congruence of two or more genes (and the absence of significant conflict in the remaining gene phylogenies) as a criterion for diagnosing phylogenetic species, multiple terminal lineages were resolved within the global *B. bassiana* complex. The arrangement of insect host orders on the tree was highly intermixed. Coding host origin as an equally weighted, non-polarized character, permutation tests revealed that the observed pattern of host association is indistinguishable from a random distribution. In contrast, evidence for continental endemism of multiple terminal lineages suggests that allopatric speciation is the principal mode of speciation in this complex. Although data from eight genes were analyzed, many terminal clades within the *B. bassiana* phylogeny failed to receive significant support. We attribute this lack of phylogenetic resolution to a historically recent phylogenetic radiation coupled with frequent intercontinental dispersals. Polymorphic microsatellite loci for *B. bassiana* s.l. have been developed and these show considerable promise as tools for defining species boundaries and for determining the underlying genetic structure of phylogenetic species.

**Contributed paper. Tuesday, 5:30.**

**Risk assessment of using mycoinsecticides: Prevalence of a commercial **Beauveria bassiana** strain and its impact on conspecific indigenous populations**

**Loesla A. Castroillo**, **Eleanor Groder**, **Seanna L. Arns**, and **John D. Vandenberg**

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The fungal pathogen **Beauveria bassiana** is widely used as a mycoinsecticide for control of several insect pests, providing a biological alternative to chemical insecticides. A key advantage for microbial control agents is their potential to replicate and persist in the environment, offering continued suppression of insect pest populations. However, exploiting this advantage is commensurate with the need to determine impact of mass releases of this fungus on non-target organisms and to assure safety and long-term efficacy. To date, no information is available on the impact of a mass-released fungal entomopathogen on conspecific indigenous populations in agricultural fields. In this study we are evaluating the effects of mass releases of a commercial formulation of *B. bassiana* strain GHA on naturally occurring conspecific strains by comparing prevalence and of genetic diversity within indigenous populations of *B. bassiana* in fields with no history of GHA treatment and in fields representing a range of GHA application histories. Soil core samples from three potato farms in Maine and two sites in New York, representing different treatments, were sampled and plated on semi-selective medium for *B. bassiana*. Single spore isolates were established from representative colony forming units and isolate colony morphology was used initially to assess diversity. Then, assays were done with sequence-characterized amplified region markers to detect presence of GHA and with random amplified polymorphic DNA and amplified fragment-length polymorphisms markers to assess genetic diversity among indigenous isolates. Preliminary data suggest the persistence of GHA in Maine sites with multiple treatments, whether continuously for the last 9 years or 5 years after the last GHA application. Strain GHA was also found to be the predominant isolate in these fields, with only a few indigenous strains present. In contrast, soil samples from an organic farm in New York, never treated with GHA, revealed a diverse array of *B. bassiana* isolates. Whether indigenous strains are displaced by continuous mass releases of *B. bassiana* GHA formulated products and whether indigenous populations recover in prevalence with time from the last spray will be determined by comparing prevalence and diversity of *B. bassiana* strains from the different test sites over time.

**Contributed paper. Tuesday, 5:45.**

**Evaluation of entomopathogenic fungi for microbial control of the greenhouse pests **Myzus persicae** and **Aphis gossypii****

**Melanie Filotas** 1, **Stephen Wraight** 1 and **John Sanderson**

1Dept. of Entomology, Cornell Univ., Ithaca, NY, USA; 2USDA Agriculture Research Service, US Plant, Soil, & Nutrition Laboratory, Tower Road, Ithaca, NY, USA

The green peach aphid, *Myzus persicae*, and the melon aphid, *Aphis gossypii*, are among the most common pests of ornamental and vegetable crops in commercial greenhouses throughout the United States. At present only one microbial insecticide, the *Beauveria bassiana*-based BotaniGard, is commercially available for use against aphids in US greenhouses. We conducted a series of laboratory assays to identify additional strains of entomopathogenic fungi which might be effective against aphid pests of greenhouse crops. Adult *M. persicae* and *A. gossypii* were exposed to spray applications of thirteen isolates of four Hyphomycete fungi (*B. bassiana*, *Lecanicillium (=Verticillium) lecanii*, *Metarhizium anisopliae* and *Paecilomyces fumosoroseus*). While adults of both species were highly susceptible to most isolates (LC50 values <100 spores/mm2 for 10 of 13 isolates), high rates of aphid reproduction were always observed prior to death. To evaluate effects of fungal infection on fecundity, adults were treated with high rates (>1000 spores/mm2) of four of the previously tested isolates, and the numbers of offspring produced prior to death were recorded. Reproduction by *M. persicae* adults was not significantly affected by any of the fungal treatments, whereas that of *A. gossypii* was significantly reduced by exposure to all isolates tested except commercial strain GHA. Nevertheless, adult *A. gossypii* were still able to increase their numbers 15 to 20 fold prior to succumbing to fungal infection, suggesting that to identify isolates capable of effectively controlling aphid populations, pathogen screening should be directed against nymphal stages. In preliminary screens using a single dose (ca. 1000 spores/mm2) of the 13 strains tested against adults, first instar nymphs proved to be less susceptible than adults, with *M. persicae* the more susceptible of the two species. However, one *B. bassiana* isolate (ARSEF 5494) was highly virulent, causing ≥80% mortality for nymphs of both species. The commercial strain, GHA, was effective against the green peach aphid but was among the least effective of the isolates tested against the melon aphid. More extensive screening of 40 isolates against nymphal stages of both species is currently underway.

**Contributed paper. Tuesday, 6:00.**

**The effects of drying on germination and activity of **Metarhizium anisopliae var. acridum** conidiospores**

**Bonifácio P. Magalhães** 1,2 and **Drion G. Boucias**

1Entomology & Nematology Dept., Univ. of Florida, Gainesville, Florida 32605, USA; 2Permanent address: Embrapa Recursos Genéticos e Biotecnologia, C.P. 2372, Brasilia, DF, Brazil

**Metarhizium anisopliae var. acridum**, isolate CG 423, is being developed as a mycoinsecticide against grasshoppers in Brazil. The shelf-life of the final product may be considerably increased by drying the conidiospores. This study was carried out to clarify the effects of drying on the germination behavior on artificial medium and activity of *M. anisopliae var. acridum* conidiospores. Conidia were produced on S. dextrusa agar amended with 1% yeast extract at 27°C with a 12h photophase and harvested 12 days after inoculation. Conidia were then transferred and kept in an Auto-desicator Cabinet (Scienceware®), Pequannock, NJ, USA). The loss of water in the drying chamber was stabilized at 0.16%/h. The last spray water (dry weight) of fresh and dried conidia were 66 and 1%. Conidia were kept in the drying chamber for 7 days and after that they were boiled dry.
properly. Conidial germination was monitored with the aid of an optical microscope (Leitz) connected to a camera (RT Monochrome, Diagnostic Instruments, Inc.). Measurements of conidia (dried and fresh) and germ tube length were taken at 0, 3, 6, 9, 12, 13, 14, 15, 16, 17 and 18h. To compare the activity of dried and fresh conidia, a bioassay was performed against third instar nymphs of *Schistocerca americana*. Insects were inoculated with the deposition of 2,000 conidia contained in a 3µl suspension on the insertion of the hind leg. The insects were transferred to cages (10/cage; 3 cages/treatment) and fed to leaves of romaine lettuce daily. Mortality was recorded every day and hemolymph of dead insects examined under the microscope. Results indicated that germination and activity were not affected by the drying process. However, there was a slight delay in swelling of dried conidia. At 0h after inoculation, conidia (dried/fresh) measured 4.8 ± 0.07 / 4.8 ± 0.06 µm. At 9h fresh conidia started to swell, measuring 5.1 ± 0.07 µm. However, dried conidia measured only 4.8 ± 0.08 µm. The swelling was conspicuous at 14h when dried/fresh conidia measured 6.3 ± 0.1 / 6.4 ± 0.09 µm. The growth of germ tubes followed similar patterns for dried and fresh conidia. At 3h and 19h germ tubes from dried/fresh conidia measured 3.0 ± 0.2 (SE) / 302 ± 0.4 and 12.7 ± 0.9 / 13.8 ± 0.7 µm. Bioassay against *S. americana* using dried and fresh conidia resulted in high mortality (>95%) 7 days after inoculation.

Contributed paper. Tuesday, 6:15.

**Potential use of *Paecilomyces fumosoroseus* for control of the Formosan subterranean termite, *Coptotermes formosanus* Shiraki**

Maureen S. Wright¹, Mark A. Jackson² and William J. Connick¹

¹USDA, Agric. Research Service, Southern Regional Research Center, New Orleans, LA, USA; ²USDA, Agric. Research Service, National Center for Agric. Utilization Research, Peoria, IL, USA

Subterranean termites are destructive pests in tropical and temperate regions throughout the world. One subterranean termite species, the Formosan subterranean termite (FST), *Coptotermes formosanus* Shiraki, is becoming the predominant termite pest species in the southern United States. The desire to develop effective, non-chemical controls for native subterranean termites and the FST has led to the investigation of various microbial biological control agents. In this study, we evaluated the use of conidia and blastospores of the entomopathogenic fungus *Paecilomyces fumosoroseus* (Pfr) as a biological control for the FST. Termite mortality, disease transmission to termite nestmates and termite repellency to spore preparations of Pfr were investigated. After 5 minutes exposure to blastospores of various Pfr strains (2.1 x 10⁸ blastospores/cm² of filter paper), a mortality rate of 100% of the FST was achieved within 9 days. To measure disease transmission, FST workers were exposed for 5 minutes to conidia of Pfr strain ARSEF 3581 and then incubated with an equal number of FST nestmates that were not directly exposed to the fungus. Transmission of *P. fumosoroseus* infections to unexposed FST nestmates resulted in 80-100% mortality for all FST after 14 days incubation. In all experiments the mortality rate of termites exposed to blastospores or conidia of Pfr were significantly higher compared to unexposed control FST populations. Repellency studies suggested that liquid culture-produced blastospores of Pfr were significantly less repellent compared to some conidial preparations of Pfr. These data show that Pfr has potential for use as a biological control agent for the FST. Large numbers of infectious blastospores of Pfr can be easily and inexpensively produced and stabilized for use as sprays or for incorporation into formulations, enhancing the potential of this fungus as a biological termiticide.
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