PROGRAM AND ABSTRACTS

36TH ANNUAL MEETING

SOCIETY FOR INVERTEBRATE PATHOLOGY

26-30 JULY 2003

BURLINGTON, VERMONT

Society for Invertebrate Pathology

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PROGRAM

2003

IMPORTANT NOTES:

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

STU indicates papers being judged for graduate student presentation awards

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**

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

Opening Ceremonies and SIP Founders' Memorial Lecture

Opening Ceremonies

John Burand, Chair, Organizing Committee
Harry Kaya, President, SIP

Founder's Memorial Lecture

Dudley Pinnock, Chair, Founders' Lecture Committee
Honoree: **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.

2:24 **Function of the peritrophic membrane in viral pathogenesis.** P Wang. Dept. of Entomology, New York State Agric. Expt. Sta., Cornell Univ., Geneva, NY, USA.

2:48 **Beet armyworm midgut gene expression correlated with sensitivity or resistance to *Bacillus thuringiensis* delta-endotoxin Cry1Ca.** RA de Maagd[1], PL Bakker[1], T Gechev[1], T-Y Man[1], S Herrero[1], WJ Moar[2]. [1]Plant Res. International, Wageningen, The Netherlands; [2]Dept. of Entomology, Auburn Univ., Auburn, AL, USA.

3:12 **Proteomic analyses of *Bacillus thuringiensis* toxin – insect midgut interactions.** MJ Adang[1,2], RJ McNall[1]. [1]Biochem. & Molecular Biology and [2]Entomology, Univ. of Georgia, Athens, GA, USA.

3:36 **Tracking the infection process of *Bacillus thuringiensis* in the insect.** C Nielsen-LeRoux[1,2], C Buisson[1], P Nel[1], M Hajaij[1], S Fedhila[1], E Guillemet[1], L Fiette[3], D Lereclus[1,2]. [1]Unité Génétique Microb. et Environ., INRA, la Minière, Gouyancourt, France; [2]Unité de Biochim. Microb. and [3]Unité d'Histotechnol. et Pathologie, Inst. Pasteur, Paris, France.

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 neoaphidis.*** PA Shah, JK Pell. Plant and Invertebrate Ecology Div., Rothamsted Research, Harpenden, Herts., UK.

3:00 **Hedgerows, flies.60.., aphids and winter survival of Entomophthorales.** J Eilenberg, C Nielsen. Dept. of Ecology, Royal Veterin. and Agric. Univ., Frederiksberg C., Denmark.

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 Solter[1], PF Solter[2], DK Pilarska[3], ML McManus[4]. [1]Illinios Natural History Survey, Urbana, IL, USA; [2]Univ. of Illinois, Veterinary Pathobiology, Urbana, IL, USA; [3]Bulgarian Acad. of Sciences, Inst. of Zoology, Sofia, Bulgaria; [4]USDA Forest Service, Hamden, CT, USA.

2:15 **Is permissiveness of *Lymantria dispar* larvae to microsporidian infections determined by the host's immune response?** G Hoch[1,2], LF Solter[1], A Schopf[2]. [1]Center for Economic Entomology, Illinois Natural History Survey, Champaign, IL, USA; [2]Inst. of Forest Entomol., BOKU–Univ. of Nat. Res. and Appl. Life Sciences, Vienna, Austria.

2:30 **STU** **Factors affecting transmission of the microsporidian, *Nosema fumiferanae,* a natural pathogen of the spruce budworm.** C Campbell[1], S Smith[1], K van Frankenhuyzen[2]. [1]Faculty of Forestry, Univ. of Toronto, Toronto, ON, Canada; [2]Great Lakes Forestry Centre, Canadian Forest Service, Natural Resources Canada, Sault Ste. Marie, ON, Canada.

2:45 **STU** **Modelling the transmission of an insect pathogen (Microsporidia) on its host, *Lymantria dispar* L. – a forest pest insect.** D Goertz[1], D Onstad[2], D Crowder[2], A Linde[1]. [1]Fachhochschule Eberswalde, Dept. of Forestry, Appl. Ecology, Alfred-Möller-Str. 1, 16225 Eberswalde, Germany; [2]Plant Sciences Lab., MC-634, Univ. of Illinois, Urbana IL, USA.

3:00 **Virulence and development of *Johenrea locustae* in two locust species: *Locusta migratoria* and *Schistocerca gregaria.*** NK Maniania[1], LJ Vaughan[2], EO Osir[1], EO Ouna[1]. [1]Internat. Centre of Insect Physiol. & Ecol. (ICIPE), Nairobi, Kenya; [2]Office of Internat. Res., Education, and Development, Virginia Polytechnic Inst. and State Univ., Blacksburg, VA, 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.

4:50 **The influence of transmission route on the epidemiology of a microsporidian parasite of *Daphnia*.** S Lass, DB Vizoso, D Ebert. Unit Ecol. and Evol., Dept. of Biol., Univ. of Fribourg, Fribourg, Switzerland.

5:20 **Population and community level effects of microsporidia in trout stream food webs.** SL Kohler[1], MJ Wiley[2]. [1]Envir. Studies Program and Dept. Biol. Sciences, Western Michigan Univ., Kalamazoo, MI, USA; [2]School 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.

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

VIRUSES – 1

Moderator: Bryony Bonning.

4:20 **Isolation and characterization of baculoviruses from greenhouse populations of *Trichoplusia ni.*** M Erlandson[1], S Newhouse[1], A Janmaat[2], K Moore[1], J Myers[2], D Theilmann[3]. [1]Agric. and Agri-Food Canada, Saskatoon Research Centre, Saskatoon, SK, Canada; [2]Dept. of Zoology, Univ. of British Columbia, Vancouver, BC, Canada; [3]Agriculture and Agri-Food Canada, Pacific Agri-Food Research Centre, Summerland, BC, Canada.

4:35 **STU** **Genotypic and phenotypic variation of *Spodoptera exempta* nucleopolyhedrovirus.** L Redman, J Cory. Molecular Ecology and Biocontrol Group, NERC-Center for Ecology and Hydrology, Oxford, UK.

4:50 **STU** **Field and safety assessment of genetically modified *Helicoverpa armigera* nucleopolyhedrovirus as a commercial insecticide.** X Sun[1,2,3], H Wang[1], X Sun[1], X Chen[1], W van der Werf[2], JM Vlak[3], Z Hu[1]. [1]Joint-Lab. of Invert. Virol. & Key Lab. of Molec. Virol., Wuhan Inst. of Virol., Chinese Acad. of Sci., Wuhan, China; [2]Crop and Weed Ecology Group and [3]Lab. 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.

5:20 **Control of false codling moth on citrus with a South African isolate of *Cryptophlebia leucotreta* granulovirus (CrleGV-SA).** SD Moore[1,2], GI Richards[1], PR Stephen[1], BA Tate[1], DA Hendry[2]. [1]Citrus Res. International, Humewood, Port Elizabeth, South Africa; [2]Dept. of Biochem., Microbiol. & Biotechnol., Rhodes Univ., Grahamstown, South Africa.

5:35 **Advances towards improving the insecticidal properties of AgMNPV.** V Romanowski[1,3], EI Arana[1], CB McCarthy[1], ME Biedma[1], A Sciocco-Cap[2], AV Goldberg[1,2], PD Ghiringhelli[3], FJR Pinedo[4], F Moscardi[5], BM Ribeiro[4]. [1]IBBM, Fac. Ciencias Exactas, Univ. Nacional de La Plata; [2]IMYZA, INTA Castelar, [3]Univ. Nacional de Quilmes; Argentina; [4]Depto. de Biologia Celular, Univ. de Brasília; [5] CNPSo-EMBRAPA, Londrina, Brazil.

5:50 **Comparing transmission between LdNPV strains: "liquefying" vs. "nonliquefying."** V D'Amico[1], J Podgwaite[1], R Webb[2], K Thorpe[2], R Fuester[3], M Valenti[4], R Pfeiffer[5], P Taylor[3], J Slavicek[6]. [1]USDA For. Serv., Hamden, CT, USA; [2]USDA, ARS, Beltsville, MD, USA; [3]USDA, ARS, Newark, DE, USA; [4]Delaware Dept. Agric., Dover, DE, USA; [5]Delaware State Univ., Dover, DE, USA; [6]USDA For. Service, Delaware, OH, USA.

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

BACTERIA – 1

Moderator: Brian Federici.

4:20 **Inheritance of resistance to *Bacillus thuringiensis kurstaki* in *Trichoplusia ni.*** AF Janmaat, J Myers. Dept. of Zool., Univ. of British Columbia, Vancouver, Canada.

4:35 **STU** **Understanding and overcoming resistance of *Plutella xylostella* to *Bacillus thuringiensis* Cry1Ac toxin.** R Gatsi[1], T Kouskoura[1], A Sayyed[2], D Wright[2], N Crickmore[1]. [1]School of Biol. Sci., Univ. of Sussex, UK; [2]Dept. of Biol. Sci., Imperial College, UK.

4:50 **STU** **Resistance to *Bacillus thuringiensis* endotoxins in the *European corn borer (Lepidoptera: Crambidae).*** H Li[1], J Gonzalez-Cabrera[2], B Oppert[3], J Ferré[2], RA Higgins[1], LL Buschman[1], KY Zhu[1], F Huang[1]. [1]Dept. of Entomol., Kansas State Univ., Manhattan, KS, USA; [2]Dept. of Genetics, Univ. of Valencia, Burjassot (Valencia), Spain; [3]Grain Marketing and Production Res. Center, USDA ARS, Manhattan, KS, USA.

5:05 **STU** **The effect of genetically modified insect-resistant *Brassica* plants on non-target invertebrates.** RE Collier[1], RH Collier[1], CC Payne[2]. [1]Horticulture Res. Internat., Wellesbourne, Warwick, UK; [2]The Univ. of Reading, Whiteknights, Reading, UK.

5:20 **STU** **Studying Cry1C-resistance mechanisms by using Sf9 cells.** D Avisar, B Sneh, N Chejanovsky[1], A Zilberstein. Dept.of Plant Science, Tel Aviv Univ., Tel Aviv, Israel; [1]Dept. of Entomology, Plant Protection Institute, Volcani Center, Bet Dagan, Israel.

5:35 **Selection with *Bacillus sphaericus* Plus Cyt1Aa from *Bacillus thuringiensis* subsp. *israelensis:* effect on *Bacillus sphaericus* resistance in mosquitoes.** MC Wirth[1], JA Jiannino[1], BA Federici[1,2], WE Walton[1]. [1]Dept. of Entomology & [2]Interdepartmental Graduate Program in Genetics, Univ. of California, Riverside, CA, USA.

5:50 **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.

Contributed Papers Sunday, 4:20-6:20. Green Mt.–C

FUNGI – 1

Moderator: Eleanor Groden.

4:20 **Isolate selection and formulation of *Beauveria bassiana* for controlling tarnished plant bug, *Lygus lineolaris* (Heteroptera: Miridae) in wild host plants.** JE Leland[1], RW Behle[2]. [1]USDA-ARS, Southern Insect Management Res. Unit, Stoneville, MS, USA; [2]USDA-ARS, National Center for Agric. Utiliz. Res., Peoria, IL, USA.

4:35 **Impact of *Beauveria bassiana* on Western tarnished plant bug.** MR McGuire. USDA-ARS, Shafter, CA, USA.

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**. MS Al-mazra'awi[1], JL Shipp[2], AB Broadbent[3], PG Kevan[1]. [1]Univ. of Guelph, ON, Canada; [2]Agric. and Agri-Food Canada, Harrow, ON, Canada; [3]Agric. and Agri-Food Canada, London, ON, Canada.

5:05 **STU** **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 Ugine[1], SP Wraight[2], JP Sanderson[1]. [1]Cornell Univ., Ithaca, NY; [2]USDA-ARS Ithaca, NY, USA.

5:20 **Evaluation of two microbial pesticides for integrated thrips control in glasshouse chrysanthemums.** EAM Beerling, D van den Berg. Appl. Plant Res. (PPO), Div. Glasshouse Horticulture, Aalsmeer, the Netherlands.

5:35 **STU** **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.

5:50 **Efficacy of *Beauveria sp.* in the control of first instar larvae of the Andean Potato Weevil (*Premnotrypes suturicallus* Kuschel).** M Kühne,[1] S Vidal,[2] K Jung,[3] D Stephan[3], A Lagnaoui[4]. [1]Internat. Potato Center, Lima, Peru; [2]Inst. for Plant Pathol. and Plant Prot., Georg-August-Univ., Göttingen, Germany; [3]Federal Biol. Res. Center for Agric. and Forestry, Inst. for Biol. Control, Darmstadt, Germany; [4]ESSD, World Bank, Washington DC, USA.

6:05 **Comparative virulence and host specificity of *Beauveria bassiana* isolates assayed against lepidopteran pests of vegetable crops.** SP Wraight[1], ME Ramos[1], JE Williams[1], PB Avery[3], ST Jaronski[2], JD Vandenberg[1]. [1]USDA-ARS, U.S. Plant, Soil, & Nutrition Lab., Tower Road, Ithaca, NY, USA; [2]Formerly Mycotech Corp, Butte, MT, USA [current address: USDA-ARS Northern Plains Agric. Res. Lab., Sidney, MT, USA]; [3]Lee Academy, Lee, ME, USA.

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

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)

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*.** J Langewald[1], NE Jenkins[2], B Ali[3], M Brüntrup[4], D Moore[2]. [1]IITA, Cotonou, Benin; [2]CABI Bioscience, Silwood Park, Ascot, UK; [3]CABI Bioscience, Caribbean and Latin America Centre, Trinidad & Tobago; [4]Freelance 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 Grzywacz[1], U Ketunuti[2], H Warburton[1]. [1]Natural Resources Inst., Univ. of Greenwich, Chatham Maritime, Kent, UK; [2]Dept. of Agric., Chatuchak, Bangkok, Thailand.

9:14 **Commercializing mycoinsecticides: The U.S. experience.** ST Jaronski. USDA REE ARS NPARL, 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.

8:25 **Luteovirus transmission barriers in aphids.** S Gray[1], F Gildow[2], D Cox-Foster[3], M Caillaud[4]. [1]USDA ARS, Dept. Plant Pathology, Cornell Univ., Ithaca, NY, USA; [2]Dept. Plant Pathology and [3]Dept. Entomology, The Pennsylvania State Univ., University Park, PA, USA; [4]Dept. Biology, Ithaca College, Ithaca, NY, 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 *Hz*SNPV in plasma of *Heliothis virescens*.** HJR 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 Monday, 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*.** R. Manasherob,[1,3] ZN Otieno-Ayayo,[1,4] E Ben-Dov,[1,3,] R Miaskovsky,[2,3] S Boussiba[2,3], A Zaritsky[1,3]. [1]Dept. of Life Sciences and [2]Microalgal Biotechnol. Lab., Ben-Gurion Univ. of the Negev, Be'er-Sheva, Israel; [3]BioSan Ltd., Ariel, Israel; [4]Dept. of Math., Envir. & Natural Sci., Solusi Univ., Bulawayo, Zimbabwe.

8:15 **Diamondback moth vs. Bt-*B. napus*/Bt-*B. rapa*: Who will win?** L Braun[1], SI Warwick[2], P Mason[2], B Zhu[3], CN Stewart Jr[4]. [1]Agric. and Agri-Food Canada, Saskatoon, Saskatchewan, Canada; [2]Agric. and Agri-Food Canada, Ottawa, Ontario, Canada; [3]Envir. Canada, National Water Res. Inst., Saskatoon, Saskatchewan, Canada; [4]Dept. 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 Bauer[1,2], DL Miller[1,] [1]USDA Forest Service, North Central Res. Station, East Lansing, MI, USA; [2]Dept. 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]. [1]Dept. of Entomology, [2]Dept. 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 Ivashov[1], AP Simchuk[1], IG Peletskaya[1], SY Gouli[2]. [1]Dept. of Ecology, V.I. Vernadsky National Univ., Simpheropol, Ukraine; [2]Entomol. Res. Lab., Univ. of Vermont, Burlington, VT, USA

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

10:00–10:30 **BREAK** Green Mt. atrium

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 Smolowitz. Marine Biological Laboratory, Woods Hole, MA, USA.

10:50 **Molecular diagnostics and phylogenetic analysis of Quahog Parasite Unknown (QPX).** NA Stokes[1], LM Ragone Calvo[1], KS Reece[2], EM Burreson[1]. [1]Dept. of Envir. & Aquatic Animal Health and [2]Aquaculture Genetics and Breeding Technology Center, Virginia Institute of Marine Science, Gloucester Point, VA, USA.

11:10 **The first occurrence of MSX disease in Canada – aberrent pathology and discovery of SSO.** SE McGladdery, 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 Ross[1], E Egbosimba[1], N Brun[1,2], M Bricelj[1], T MacRae[1,2], J Harding[3], C Couturier[3], J Parsons[3]. [1]National Res. Council, Inst. for Marine Biosciences, Halifax, NS, Canada; [2]Dept. of Biology, Dalhousie Univ., Halifax, NS, Canada; [3]Fisheries and Marine Inst. of Memorial Univ. of Newfoundland, St. John's, NF, Canada.

10:50 **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.

12:10 **Calcinosis: a new disease in lobsters, *Homarus americanus*.** ADM Dove[1], CP LoBue[2], PR Bowser[3]. [1]Dept. of Microbiol. and Immunol., Cornell College of Veter. Med.. C/o Marine Sci. Res. Center, Stony Brook Univ.; [2]NY Dept. of Envir. Conservation; [3]Dept. of Microbiol. & Immunol., Cornell Coll. of Veter. Medicine.

Contributed Papers Monday, 10:30-12:15. Lake Champlain

NEMATODES

Moderator: Albrecht Koppenhöffer.

10:30 **STU** **Genomic fingerprinting of *Xenorhabdus* spp. using repetitive sequences and PCR.** HL Smith[1], BJ Adams[1], JB Jones[2], FJ Louws[3]. [1]Dept. of Entomology and Nematology, [2]Dept. of Plant Pathol., Univ. of Florida, Gainesville, FL, USA; [3]Dept. of Plant Pathol., North Carolina State Univ., Raleigh, NC, USA.

10:45 **Evaluation of entomopathogenic nematode strains for the control of *Anoplophora glabripennis*.** D Fallon[1], L Solter[1], M Keena[2], J.Cate[3], M McManus[2], L Hanks[4]. [1]Illinois Natural History Survey, Univ. of Illinois, NSRC, Urbana, IL, USA; [2]USDA Forestry Service, Northeastern Research Station, Hamden, CT, USA; [3] Integrated Biocontrol Systems, Inc., Aurora, IN, USA; [4]Entomology Dept., Univ. of Illinois, Urbana, IL, USA.

11:00 **Susceptibility of the European crane fly to four entomopathogenic nematodes (Steinernematidae and Heterorhabditidae).** L Simard[1], G Bélair[2], J Dionne[3]. [1]Centre de Recherche en Horticulture, Univ. Laval, Québec, Canada; [2]Agric. and Agri-Food Canada, St-Jean-sur-Richelieu,

Québec, Canada; [3]Dept. of Plant Agric., Univ, of Guelph, Guelph, Ontario, Canada.

11:15 ***Steinernema scarabaei*: ecology and efficacy against white grubs.** AM Koppenhöfer, E.M. Fuzy. Dept. of Entomol., Rutgers Univ., New Brunswick, NJ, USA.

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]. [1]Dept. of Entomology, Ohio State Univ., Ohio Agric. 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, *Steinernema 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 *Steinernema* spp. in *Diabrotica undecimpunctata howardi*.** ME Barbercheck[1]*, J Wang[1], C Brownie[2]. [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 **STU** **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[2], L King[1]. [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[2]. [1]Dept. of Agronomy, Univ. Federal do Parana, Curitiba, PR, Brazil; [2]Embrapa Soja, Londrina, PR, Brazil.

11;00 **Trends of mass production of microbial pesticides in Russia.** MV Shternshis[1], VV Gouli[2]. [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[2], I.T. De Ley[2], and M. Hoddle[3]. [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 **STU** **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.

12:30–2:00 **LUNCH** Adirondack Ballroom

Monday, 2:00-4:00. Adirondack Ballroom

POSTERS – 1

Posters should be displayed from Sunday UNTIL NO LATER THAN 1:00 pm, WEDNESDAY

FUNGI

F-1 ***Cordyceps staphylinidaecola*, its *Beauveria* anamorph from Korea, and neotypification of *Beauveria bassiana*.** J-M Sung[1], RA Humber[2]. [1]Dept. of Agricultural Biology, Kangwon National Univ., Chuncheon, Rep. of Korea; [2]USDA-ARS Plant Soil & Nutrition Lab., Ithaca, NY, USA.

F-2 **STU** **The specificity analyze of entomopathogenic fungus *Beauveria amorpha*.** D Yaginuma, H Hiromori, M Hatsukade. Dept. of Applied Entomol., Fac. of Agriculture, Shizuoka Univ., Ohya836, Shizuoka 422-8529, Japan

F-3 ***Beauveria* as a possible coffee endophyte.** F.J. Posada, F.E. Vega, S.A. Rehner. Insect Biocontrol Laboratory, USDA, ARS, BARC-W, Belstville, MD, USA.

F-4 **Comparative pathogenicity and genetic variation of *Beauveria bassiana* isolates from Asian longhorned beetle and other cerambycids.** LS Bauer[1,2], H Liu[1], DL Miller[2], LA Castrillo[3], JD Vandenberg[3]. [1]Dept. of Entomol., Michigan St. Univ., East Lansing, MI, USA; [2]USDA Forest Service, North Central Research Station, East Lansing, MI, USA; [3]USDA Agric. Res. Serv., Ithaca, NY, USA.

F-5 **Efficacy of *Beauveria sp.* in the control of adult Andean Potato Weevil (*Premnotrypes suturicallus* Kuschel).** M Kühne[1], S Vidal[2], K Jung[3], D Stephan[3], A Lagnaoui[4]. [1]International Potato Center, Lima, Peru; [2]Institute for Plant Pathology and Plant Protection, Georg-August-Univ., Göttingen, Germany; [3]Federal Biol. Res. Center for Agric. and Forestry, Institute for Biological Control, Darmstadt, Germany; [4]ESSD, The World Bank, Washington DC, USA.

F-6 **Genetic recombination among vegetatively compatible strains of *Beauveria bassiana* in a susceptible insect host.** LA Castrillo[1], JD Vandenberg[2], MH Griggs[2]. [1]Dept. of Entomology, Cornell Univ., Ithaca, NY, USA; [2]USDA-ARS, US Plant, Soil & Nutrition Lab., Ithaca, NY, USA.

F-7 **Comparative virulence of wild type and recombinant vegetatively compatible strains of *Beauveria bassiana* against Colorado potato beetle.** JD Vandenberg[1], LA Castrillo[2], MH Griggs[1], SL Annis[3], E Groden[3]. [1]USDA-ARS, US Plant, Soil & Nutrition Lab., Ithaca, NY, USA; [2]Dept. of Entomol., Cornell Univ., Ithaca, NY, USA; [3]Dept. Biol. Sci., Univ. of Maine, Orono, ME, USA.

F-8 **STU** **Horizontal transmission of *Beauveria bassiana* between cadavers and adults of *Leptinotarsa decemlineata*.** E Klinger, E Groden. Dept. of Biological Sciences, Univ. of Maine, Orono, Maine, USA.

F-9 **STU** **The impact of scavenging insects on disease persistence in Colorado potato beetle populations.** KL Coluzzi, E Groden, F Drummond. Dept. of Biological Sciences, Univ. of Maine, Orono, ME, USA.

F-10 **STU** **The intraguild interactions of the greenhouse whitefly predator *Dicyphus hesperus* with the entomopathogen *Beauveria bassiana*.** RM Labbé[1], C Cloutier[2], J Brodeur[1]. [1]Dépt. de phytologie, Univ. Laval, Québec, QC, Canada; [2]Dépt. de biologie, Univ. Laval, Québec, QC, Canada.

F-11 **Interactions between impatiens pollen, *Beauveria bassiana* and adult female Western flower thrips (*Frankliniella occidentalis*).** TA Ugine[1], SP Wraight[2],

JP Sanderson[1]. [1]Cornell Univ., Ithaca NY, USA; [2]USDA-ARS Ithaca, NY, USA.

F-12 **Comparation techniques and parameters used in compatibility tests between *Beauveria bassiana* and chemical pesticides *in vitro*.** PMOJ Neves, RZ Silva. Depto. Agronom., Univ. Estadual de Londrina, PR, Brazil.

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

F-14 **Molecular characterization and comparative virulence of *Beauveria bassiana* isolates for control of the shore fly, *Scatella stagnalis*, on greenhouse crops.** M. Filotas[1], L. Castrillo[1], S. Wraight[2], J. Vandenberg[2], J. Sanderson[1]. [1]Dept. of Entomology, Cornell Univ., Ithaca, NY, USA; [2]USDA Agriculture Research Service, US Plant, Soil, & Nutrition Lab., Ithaca, NY, USA.

F-15 **Occurrence of Hyphomycete fungi from natural birch habitats and eroded land in sub-arctic Iceland and Faroe Islands.** C Nielsen[1], C Wolsted[1], S Harding[1], E Oddsdóttir[2,3], G Halldórsson[2], T Leivsson[4], R Sen[3], J Eilen-berg[1]. [1]Dept. of Ecology, The Royal Veterinary and Agric. Univ., Denmark; [2]Iceland Forest Research, Iceland; [3]Dept. of Biosciences, Univ. of Helsinki, Finland; [4]Forestry Service of the Faroe Islands, Faroe Islands.

F-16 **Biological control of weevils and scarabs in greenery and Christmas tree plantations.** S Vestergaard, C Nielsen, S Harding, J Eilenberg. Royal Veterinary and Agric. Univ., Dept. of Ecol., Zoology Section, Frederiksberg C, Denmark.

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-18 **Characterization of *in vitro* destruxin production, pathogenicity, and RFLP patterns of peptide synthetase genes in *Metarhizium anisopliae*.** ACL Churchill[1], S Krasnoff[2], Y-S Moon[1], H McLane[1], J Williams[2], J Vandenberg[2], D Gibson[2]. [1]Boyce Thompson Inst. for Plant Res., and [2]USDA-ARS Plant Protection Res. Unit, US Plant, Soil, & Nutr. Lab., Ithaca, NY, USA.

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

F-20 STU **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. [1]Dept. of Biology, Utah State Univ., Logan, Utah, USA.

F-21 **Relative performances of fungal pathogens isolated from acridid hosts collected in desert locust habitats in Eastern Ethiopia.** T Megenasa[1], L Vaughan[2], E Seyoum[3], E Samuel[1]. [1]Desert Locust Control Organization for Eastern Africa (DLCO-EA), Addis Ababa, Ethiopia; [2]Virginia Polytechnic Inst. and State Univ., Blacksburg, Virginia, USA; [3]Dept. of Biology, Univ. of Addis Ababa, Ethiopia.

F-22 Efficacy of locally collected isolates of *Metarhizium anisopliae* var *acridum* and *Metarhizium flavoviride* on three acridid pests in Senegal, West Africa. A Niassy[1], K Badji[1], L Vaughan[2]. [1]Direction de la Protection des Végétaux, Dakar, Senegal; [2]Office 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 regime. J Fargues[1], N Smits[1], C Vidal[1], W Meikle[2], G Mer, N Issaly[3], L Vaughan[4]. [1]UMR, Centre de Biologie et de Gestion des Populations, INRA, Montpellier, France; [2]European Biol. Control Lab., USDA-ARS, Montferrier, France; [3]UMR, Microbiol., INRA, Dijon, France; [4]Office of International Research and Development, Virginia Tech, Blacksburg, VA, USA.

F-24 **Influence of culture conditions, nutrition, oxygen supply and pH on production of blastospores of *Metarhizium anisopliae* var. *acridum* in submerged fermentation.** N Issaly[1], H Chauveau[1], F Aglevor[2], J Fargues, A Durand[1], L Vaughan[2], F Vega[3], G Mercadier[3], C Quimby[3]. [1]INRA, UMR, Microbiologie, Dijon, France; [2]Office of Internat. Research, Educ. and Develop.,Virginia Tech, Blacksburg, VA USA; [3]INRA, UMR, Centre de Biologie et de Gestion des Populations; [4]USDA-ARS, European Biol. Control Lab., Montferrier-sur-Lez, France.

F-25 **Development of potential *Metarhizium* biocontrol agents: insights from molecular data.** MC Bon[1], C Hurard[1], PC Quimby[1], J Fargues[2], W Meikle[1], G Mercadier[1], L Vaughan[3]. [1]European Biol. Control Lab., USDA-ARS, and [2]Centre de Biologie et de Gestion des Populations, Montferrier sur Lez, France; [3]Office of Internat. Res., Education 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[1,2], J Faull[1], M Simmonds[3]. [1]School of Biol. and Chem. Sciences, Birkbeck College, London, UK; [2]Lee Academy, Lee, ME, USA; [3]Royal 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[1,2], J Faull[1], M Simmonds[3]. [1]School of Biological and Chemical Sciences, Birkbeck College, London, UK; [2]Lee Academy, Lee, ME, USA; [3]Royal Botanic Gardens, Kew, Richmond, Surrey, UK.

F-28 STU **Zn dependence of growth and dipicolinic acid production in *Paecilomyces fumosoroseus*.** A Asaff[4], C Cerda[2], M de la Torre[2], G Viniegra[1]. [1]Universidad Autónoma Metropolitana, México D.F., México; [2]Centro de Investigación y de Estudios Avanzados del IPN, México D.F., México.

F-29 **Phylogenetic relationships of entomopathogenic fungi based on mitochondrial SSU rDNA sequences.** DR Sosa-Gomez[1], KT Hodge[2], RA Humber[3], E Binneck[1]. [1]Embrapa Soja, Londrina, PR, Brazil; [2]Dept. of Plant Pathology, Cornell Univ., Ithaca, NY, USA; [3]USDA-ARS Plant Soil & Nutrition Lab., Ithaca, NY, USA.

F-30 STU **Field incidence of *Nomuraea rileyi* and evidence that multiple strains are present in the same field.** Ö 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, Shizuoka Univ., Shizuoka, Japan.

F-32 **Analysis of the chitinase gene of the dimorphic mycopathogen, *Nomuraea rileyi*.** R Wattanalai[1], D Boucias[2], A Tartar[2], C Wiwat[1]. [1]Dept. of Microbiol., Fac. of Pharmacy, Mahidol Univ., Bangkok, Thailand; [2]Dept. of Entomol. & Nematol., Univ. Florida, Gainesville, FL, USA.

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 Kim[1], DJ Im[1], KC Kim[2], DR Choi[1], DW Roberts[3]. [1]Div. of Entomol., NIAST, RDA, Korea; [2]Dept. of Agrobiol., Chonnam National Univ., Korea; [3]Dept. Biology, Utah State Univ., USA.

F-35 **Comparison of Japanese and American isolates of *Entomophaga maimaiga.*** S Thomas, J Elkinton. Dept. of Entomology, Univ. of Massachusetts, Amherst, MA, USA.

F-36 **STU** **Influence of insecticide treatments, irrigation, and Bt cotton on population dynamics of the cotton aphid, *Aphis gossypii* Glover and its pathogenic fungus, *Neozygites fresenii* (Zygomycetes: Entomophthorales).** R Anwar, GR Carner. Dept. of Entomol., Clemson Univ., SC, USA.

F-37 **Do *Vicia faba* plants use the aphid pathogen *Pandora neoaphidis* as a bodyguard?** J Baverstock[1,2], PG Alderson[2], SL Elliot[3], JK Pell[1]. [1]Plant and Invertebrate Ecology Division, Rothamsted Research, UK; [2]Division of Agricultural Sciences, The Univ. of Nottingham, UK; [3]NERC Centre for Population Biology, Imperial College, Silwood Park, UK.

F-38 ***In vitro* interactions between two fungal pathogens of *Plutella xylostella*: *Pandora blunckii* and *Zoophthora radicans.*** A Guzman Franco[1,2], PG Alderson[2], JK Pell[1]. [1]Plant and Invertebr. Ecology Division, Rothamsted Research, Harpenden, UK; [2]Division of Agricultural Sciences, Univ. of Nottingham, UK.

F-39 **Analysis of EST sequences from the entomopathogenic alga *Helicosporidium* sp. (Chlorophyta, Trebouxiophyceae).** A Tartar[1], PJ Keeling[2], DG Boucias[1]. [1]Dept. of Entomol. & Nematol., Univ. Florida, Gainesville, USA; [2]Dept. Botany, Univ. of Brit. Columbia, Vancouver, Canada.

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

MICROSPORIDIA & PROTOZOA

MP-1 **STU** **Influences of Dimilin® on a microsporidian isolate of the genus *Nosema.*** D Goertz, A Linde. Fachhochschule Eberswalde, Dept. of Forestry, Appl. Ecol., Eberswalde, Germany.

MP-2 **Characteristics of a microsporidian parasite, *Vairimorpha kyonggii* n. sp. isolated from *Helicoverpa armigera*.** H Iwano[1], S Akutsu[2], T Hukuhara[1]. [1]College of Biores. Sci., Nihon Univ., Fujisawa, Kanagawa, Japan: [2]Kanagawa-Ken Plant Protection Office, Hiratsuka, Kanagawa, Japan.

MP-3 **Occurrence of pathogens in bark beetles (Coleoptera, Scolytidae) from Alpine pine (Pinus cembra L.).** U Händel, R Wegensteiner. Institute of Forest Entomol., Forest Pathology and Forest Protection, BOKU–University of Nat. Resources and Appl. Life Sciences, Vienna, Austria.

MP-4 **STU** **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 Streett[1], E Villavaso[2]. [1]USDA-ARS, Biol. Control and Mass Rearing Res. Unit, Mississippi State, MS, USA; [2]USDA-ARS, Southern Insect Management Research Unit, Mississippi State, MS, USA.

MICROBIAL CONTROL

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 Meikle[1], G Mercadier[1], AA Kirk[1], M-C Bon[1], L Sawicki[1], F Derouané[1], A Peppuy[2], Y He[3], A Reid[4], PC Quimby[1]. [1]European Biological Control Lab, USDA - ARS, Campus International de Baillarguet, Montferrier sur Lez, St. Gely du Fesc., France; [2]Observatoire Régional de Lutte Anti-Termites (ORLAT), St. Andre, La Réunion ; [3]Lab. of Insect Ecol., South China Agric. Univ., Wushan, Guangzhou, China; [4]Edinburgh, Scotland, UK.**

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

MC-4 **Heritability and plasticity of immune function in the Egyptian cotton leafworm.** SC Cotter[1,2], K Wilson[1]. [1]Institute of Biological Sciences, Univ. of Stirling, Stirling, UK; [2]CSIRO 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.

NEMATODES

N-1 **Entomopathogenic nematode delivery systems for biological control of pests on major outdoor crops: the case of oilseed rape.** I Menzler-Hokkanen, HMT Hokkanen. Lab. of Applied Zoology, Univ. of Helsinki, Finland.

N-2 **Conservation of entomopathogenic nematode populations through the manipulation of crop diversity in vegetable production systems.** J Lawrence, C Hoy, P Grewal. Dept. of Entomology, Ohio Agric. Res. and Develop. Center, The Ohio State Univ., Wooster, OH, USA.

N-3 **Dispersal of entomopathogenic nematodes incorporated into a stochastic and spatially explicit model of population dynamics.** CW Hoy, J Lawrence, P Grewal. Dept. of Entomology, The Ohio State Univ., Ohio Agricultural Res. and Develop. Center, Wooster, Ohio, USA.

N-4 Low cost liquid fermentation of entomopathogenic nematodes. **N Pye[1], E Carvalho[1], W Curtis[2], A Pye[1]. [1]BioLogic Company, Willow Hill, PA, USA; [2]The Pennsylvania State Univ., University Park, PA, USA.**

N-5 **Field survey and evaluation of entomopathogenic nematodes for white grub *Phyllophaga vetula* (Horn) control in Oaxaca, Mexico.** J Ruiz-Vega[1], T Aquino-Bolaños[1], HK Kaya[2], P Stock[2]. [1]CIIDIR OAX., Sta. Cruz

Xoxocotlán, Oax., México; [2]Univ. of California, Dept. of Nematology, Davis, CA, USA.

N-6 STU **Evasive behavior of white grub species against entomopathogenic nematodes.** CA Yoder, PS Grewal. Dept. of Entomol., Ohio State Univ., OARDC, Wooster, OH, USA.

N-7 **New strains of the entomopathogenic nematode, *Steinernema riobrave*: are they better for biological control of the citrus root weevil, *Diaprepes abbreviatus*?** RJ Stuart[1], D Shapiro-Ilan[2], CW McCoy[1]. [1]Univ. of Florida, CREC-IFAS, Lake Alfred, FL, USA; [2]USDA-ARS, Southeast Fruit and Tree Nut Res. Lab., Byron, GA, USA.

N-8 **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.

Contributed Papers — Monday, 2:00-3:45. Green Mt.-A

BACTERIA – 3

Moderator: Vincent D'Amico.

2:00 STU **Pore-forming properties of *Bacillus thuringiensis* insecticidal toxin Cry9Ca mutants in the insect midgut brush border membrane.** J-F Brunet[1,2], V Vachon[1,2], G Arnaut[3], J Van Rie[3], J-L Schwartz[1,2,4], R Laprade[1,2]. [1]Groupe d'étude des protéines membranaires, Univ. de Montréal, Montreal, Quebec, Canada; [2]Biocontrol Network; [3]Bayer CropScience, Ghent, Belgium; [4]Biotechnology Res. Inst., National Res. Council, Montreal, Quebec Canada.

2:15 STU **Differential effects of pH and ionic strength on the pore-forming activity of *Bacillus thuringiensis* toxins.** M Fortier[1,2], M Kirouac[1,2], V Vachon[1,2], O Peyronnet[1,2], J-L Schwartz[1,2,3], R Laprade[1,2]. [1]Groupe d'étude des protéines membranaires, Univ. de Montréal, Montreal, Quebec, [2]Biocontrol Network, and [3]Biotechnology Res. Institute, National Res. Council, Montreal, Quebec, Canada.

2:30 STU **Mutations in domain I interhelical loops affect the rate of pore formation by the *Bacillus thuringiensis* Cry1Aa toxin in insect midgut membrane vesicles.** G Lebel[1,2], V Vachon[1,2], G Préfontaine[2,3], L Masson[2,3], F Girard[1,2], M Juteau[1,2], A Bah[2,3], B Rancourt[4], CVincent[4], R Laprade[1,2], J-L Schwartz[1,2,3]. [1]Groupe d'étude des protéines membranaires, Univ. de Montréal, Montreal, Quebec, [2]Biocontrol Network, [3]Biotechnol. Res. Inst., National Res. Council, Montreal, Quebec; [4]Horticult. Res. and Developm. Centre, Agric. and Agri-Food Canada, Saint-Jean-sur-Richelieu, Quebec, Canada.

2:45 STU **Role of a-helix 4 in the *Bacillus thuringiensis* Cry1Aa toxin: cysteine scanning mutagenesis.** F Girard[1,2], G Préfontaine[2,3], V Vachon[1,2], Y Su[1,2], L Marceau[1,2], A Bah[2,3], L Masson[2,3], J-L Schwartz[1,2,3], R Laprade[1,2]. [1]Groupe d'étude des protéines membranaires, Univ. de Montréal, Montreal, Quebec, [2]Biocontrol Network, and [3]Biotechnology Res. Inst., National Res. Council, Montreal, Quebec, Canada.

3:00 ***Cyt1Ca*—a new *Bacillus thuringiensis* subsp. *israelensis* gene: cloning, purification and characterization of the encoded toxin.** R Manasherob[1], M Itsko[1], N Baranes[1], E Ben-Dov[1], S Boussiba[2], C Berry[3], A Zaritsky[1]. [1]Dept. of Life Sci., and [2]Microalgal Biotechnol. Lab, Ben-Gurion Univ. of the Negev, Be'er-Sheva, Israel; [3]Cardiff Univ., School of Biosciences, Cardiff,, Wales, UK.

3:15 **Molecular genetic analysis and enhancement of Cry19A synthesis in *Bacillus thuringiensis.*** JE Barboza-Corona[1,3], H-W Park[1], BA Federici[1,2]. [1]Dept. of Entomol. and [2]Graduate Programs in Genetics and Microbiol., Univ. of California, Riverside, CA, USA; [3]Instituto de Ciencias Agrícolas, Univ. de Guanajuato, Irapuato, Guanajuato, México.

3:30 ***Cyt1A* synergizes toxicity of Bs Bin by enhancing its insertion through the mosquito midgut microvillar membrane.** BA Federici[1,2], MC Wirth[1], JJ Johnson[1], H-W Park[1], DK Bideshi[1], WE Walton[1]. [1]Dept. of Entomology and [2]Interdepartmental Grad. Progr. in Genet. and Microbiol., Univ. of California, Riverside, CA, USA.

4:00–4:30 **BREAK** Green Mt. atrium

Symposium (Cross-Div.) — Monday, 4:30–6:40. Green Mt.-A

Host altered behavior: Host mediated or pathogen induced

Organizer/Moderator: Helen Roy

4:30 **Changes in host behaviour: host altered or pathogen induced?** HE Roy. Envir. Sciences Res. Centre / Dept. of Life Sciences, Anglia Polytechnic Univ., Cambridge, UK.

4:40 **Manipulation of host behavior by entomopathogenic fungi.** AE Hajek, JE Losey, C Gilbert. Dept. of Entomology, Cornell Univ., Ithaca, NY, USA.

4:59 **Host manipulation by insect baculoviruses.** JS Cory. Molecular Ecology and Biocontrol Group, NERC Centre for Ecology & Hydrology, Oxford, UK.

5:18 **Alteration of host physiology and mating behavior resulting from virus replication.** JP Burand. Depts. of Entomology and Microbiology, Univ. of Massachusetts, Amherst, MA, USA.

5:37 **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.

5:56 **Disease resistance in crowds, density-dependent prophylaxis in the Egyptian armyworm.** SC Cotter[1], RS Hails[2], JS Cory[2], K Wilson[1]. [1]Institute Biol. Sci., Univ. of Stirling, Stirling, UK; [2]NERC Centre for Ecol. & Hydrol., Oxford, UK.

6:15 **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.

Contributed Papers — Monday, 4:30-5:45. Lake Champlain

PROTOZOA and ALGAE

Moderator: Drion Boucias.

4:30 **Prevalence of eukaryotic gut parasites in *Drosophila* along an urban gradient.** MA Ebbert, J. Avondet, R. Blair. Dept. of Zoology, Miami Univ., Oxford, OH, USA.

4:45 **Bethylid parasitoids of grain beetles are vectors and potential reservoirs of *Mattesia oryzaephili.*** J Lord. GMPRC, USDA-ARS, Manhattan, KS, USA.

5:00 **Action of *Malamoeba scolyti* Purrini (Rhizopoda, Amoebidae) in different bark beetle hosts (Coleoptera, Scolytidae).** J-F Kirchhoff[1], R Wegensteiner[2], J Weiser[3],

E Führer[2]. [1]Wegberg, Germany; [2]Instit. of Forest Entomology, Forest Pathol. and Forest Protection, BOKU - Univ. of Natural Resources and Applied Life Sciences, Vienna Austria; [3]Insect Pathology, Instit. of Entomology, Czech Acad. of Sciences, Ceske Budejovice, Czech Rep.

5:15 STU ***In vitro* development of *Helicosporidium*.** M Botts, S Shapiro, J Becnel, D Boucias. Dept. of Entomology and Nematology, Univ. of Florida, Gainesville, FL, USA.

5:30 **Influence of *Helicosporidium* spp. infection on development and survival of three noctuid species.** V-U Blaeske, DG Boucias. Dept. of Entomology and Nematology, Univ. of Florida, Gainesville, FL, USA.

Contributed Papers Monday, 4:30-6:15. Green Mt.-C

VIRUSES – 2

Moderator: James Slavicek.

4:30 **Origins of replication in *Cydia pomonella* granulovirus.** S Hilton, D Winstanley. Pest Control Strategies, Horticulture Res. Internat., Wellesbourne, Warwickshire, UK.

4:45 STU **Formation of budded virus at the plasma membrane in baculovirus-infected cells involves the localisation of gp64 within lipid rafts.** FJ Haines[1], AL Patmanidi[1], CR Hawes[1], RD Possee[2], LA King[1]. [1]School of Biol. and Molec. Sci,, Oxford Brookes Univ., Gipsy Lane Campus, Oxford, UK; [2]NERC Inst. of Virology and Environmental Microbiology (CEH, Oxford), Oxford, UK.

5:00 STU **RNA interference in uninfected and baculovirus-infected lepidopteran cells.** TI Zaki[1,3], JE Maruniak[1,2]. [1]Dept. of Microbiology and Cell Science and [2]Dept. of Entomol. and Nematol., Univ. of Florida, Gainesville, Florida, USA; [3]Agricultural 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 Popham[1]. USDA Forest Serv., Forestry Sci. Lab., Delaware, OH, USA; [1]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., ARO, The Volcani Center, Bet Dagan, Israel.

5:45 **Transcriptional regulation of a *Chilo iridescent* virus early and late gene.** R Nalçacioglu[1,2], Z Demirbag[2], JM Vlak[1], MM van Oers[1]. [1]Lab. of Virology, Wageningen Univ., The Netherlands; [2]Dept. 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.

Monday, 6:45-7:45 pm
DIFFERENT START TIMES MAY BE ANNOUNCED!

SIP Division Business Meetings:

Fungi Green Mountain–A
Nematodes Green Mountain–C

8:30-10:30 pm
Microsporidia Green Mountain–B

Workshop following business meeting at approx. 9:00p:
Molecular phylogeny and the classification of the Microsporidia.
(C. Vossbrinck, organizer/speaker)

DINNER
Enjoy exploring Burlington's many possibilities!

TUESDAY - 29 July

Symposium (Cross-Div.) Tuesday, 8:00–10:00. Green Mt.–A

You are what you eat: Multitrophism in invertebrate pathology systems

Organizers: Kelli Hoover, Gary Felton, Patricia Stock.
Moderators: Kelli Hoover, Gary Felton.

8:00 **Plant mediation of bacterial disease and lethality in insects.** GW Felton[1], I Ali[2], S Young[2]. [1]Dept. of Entomol., Penn State Univ., University Park, PA, USA; [2]Dept. of Entomology, Univ. of Arkansas, Fayetteville, AR, USA.

8:20 **Influence of transgenic BT plants on the performance of *Macrocentrus cingulum*, a parasitoid of *Ostrinia nubilalis*.** SL Sked, DD Calvin, C De Moraes, N Ostiguy. Dept. of Entomol., Pennsylv, State Univ., University Park, PA, USA.

8:40 **The influence of host plant on the ecology of insect-baculovirus interactions.** JS Cory. Molec. Ecol. and Bio-control Grp., NERC Centre for Ecol. & Hydrol., Oxford, UK.

9:00 **Plant-mediated inhibition of disease caused by baculo-viruses.** 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 Koppenhöfer. Dept. of Entomology, Rutgers Univ., New Brunswick, NJ, USA.

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

Symposium (Div. of Nematodes) Tuesday, 8:00-10:00. Green Mt.–C

Genomics of entomopathogenic nematode-bacterium complexes

Organizers/Moderators: Patricia Stock, Parwinder Grewal.

8:00 Introduction. P. Stock.

8:10 **EPN genomics: a suggestion for and the prospects of a genome-wide analysis of EPN dauer regulatory genes by using tools of molecular genetics elaborated in *C. elegans*.** A Fodor. Dept. Genetics, Eötvös Univ., Budapest, Hungary.

8:35 **Revealing the stress tolerance mechanisms in entomo-pathogenic nematodes: a genomic approach.** I Glazer, T Zitman-Gal, H Koltai. Depts. of [1]Nematol. and [2]Genomics & Bioinformatics, The Volcani Center, Bet Dagan, Israel.

9:00 **Sticking and swarming in *Xenorhabdus nematophila*.** S Forst, H He, D-J Kim. Dept. of Biological Sciences, Univ. of Wisconsin, Milwaukee, WI, USA.

9:25 **Negotiating mutualism between *Xenorhabdus nematophila* and *Steinernema carpocapsae*.** H Goodrich-Blair,

EC Martens, K Heungens, CE Cowles, EI Vivas. Dept. of Bacteriology, Univ. of Wisconsin, Madison, WI, USA.

9:50 Conclusions and final remarks. P Stock, P Grewal.

Contributed Papers Tuesday, 8:00-10:15. Lake Champlain

FUNGI – 2

Moderator: John Vandenberg.

8:00 STU **Integration of *Metarhizium anisopliae* (Deuteromycota: Hyphomycetes) and cover crops for controlling sugar-beet root maggot (Diptera: Otitidae).** A Majumdar[1], MA Boetel[1], ST Jaronski[2], RJ Dregseth[1], AJ Schroeder[1]. [1]Entomology Dept., North Dakota State Univ., Fargo, ND, USA; [2] USDA-REE-ARS-NPARL, Sidney, MT, USA.

8:15 **Ecological role of the large nettle aphid, *Microlophium carnosum*, as an early season source of *Pandora neoaphidis*.** PA Shah, SJ Clark[1], JK Pell. Plant and Invertebrate Ecology Division and [1]Bioinformatics Unit, Rothamsted Research, Harpenden, Hertfordshire, UK.

8:30 **Tritrophic interactions between *Pandora neoaphidis*, three aphid species and different host plant resources.** PA Shah, SJ Clark[1], JK Pell. Plant and Invertebrate Ecology Division and [1]Bioinformatics Unit, Rothamsted Research, Harpenden, Herts, UK.

8:45 STU **Mycoinsecticide for stored product pest control.** A Kassa[1], D Stephan[2], S Vidal[1], G Zimmermann[2]. [1]Inst. for Plant Pathol. and Plant Protection, Entomology Section, Georg-August-univ., Goettingen; [2]Federal Biol. Res. Center for Agric. and Forestry, Inst. for Biol. Control, Darmstadt, Germany.

9:00 **Use of *Beauveria bassiana* and its environmental effects in microbial control of *Monochamus alternatus*.** M Shimazu, H Sato, N Maehara. Forestry and Forest Products Res. Institute, Tsukuba, Ibaraki, Japan.

9:15 **Entomopathogenic fungi and the emerald ash borer.** H Liu[1], LS Bauer[1,2], DL Miller[2]. [1]Dept. of Entomol., Michigan State Univ., East Lansing, MI, USA; [2]USDA Forest Service, North Central Res. Station, East Lansing, Michigan, USA.

9:30 **Impact of different components of entomopathogenic fungi on the hemlock woolly adelgid, *Adelges tsugae* Annand (Homoptera: Adelgidae).** S Gouli, W Reid, V Gouli. Entomology Research Laboratory, Univ. of Vermont, Burlington, VT, USA.

9:45 STU **Effect on bloodmeal size and egg production of the malaria mosquito *Anopheles gambiae s.s.*, when infected with *Metarhizium anisopliae*.** E-J Scholte, BGJ. Knols, W Takken. Lab. of Entomol., Wageningen Univ. & Research Centre, Wageningen, the Netherlands.

10:00 **Why fat bees get chalkbrood.** RR James[1], J Buckner[2]. [1]USDA-ARS Bee Biology & Systematics Lab, Logan, UT, USA; [2]USDA-ARS Red River Valley Agric. Res. Center, Fargo, ND, USA.

Contributed Papers Tuesday, 8:00-10:00. Green Mt.–B

VIRUSES – 3

Moderator: Dwight Lynn.

8:00 **The infection and pathogenesis of the *Amsacta moorei* entomopoxvirus in *Lymantria dispar* larvae.** B Arif[1], Q Li[2], L Pavlik[1], R Moyer[2]. [1]Lab. for Molec. Virology, Great Lakes Forestry Centre, Sault Ste. Marie, ON, Canada; [2]Dept. of Molec. Genetics and Microbiol., Univ. of Florida College of Medicine, Gainesville, FL, USA.

8:15 Identification of a novel baculovirus gene required for oral infectivity of insects: *Pif-2*. **A Pruijssers, GP Pijlman, JM Vlak. Lab. of Virology, Wageningen Univ, Wageningen, The Netherlands.**

8:30 STU **Is PIF quantity regulated by *Spodoptera littoralis* nucleopolyhedrovirus (SpliNPV)?** S Gutiérrez[1], O Simón[2], P Caballero[2], M López-Ferber[1]. Lab. de Pathologie Comparée, INRA/CNRS/UM2, St-Christol-lez-Ales, France; [2]Depto. de Producción agraria, ETSIA, Univ. Pública de Navarra, Campus de Arrosadía s/n, Pamplona, Spain.

8:45 STU **Site-directed mutagenesis of structural (VP) proteins of *Junonia coenia* densovirus (*Jc*DNV): Impact on virus morphogenesis and infectivity.** A Abd-Alla[1,2], FX Jousset[1], G Fédière,[3], M Bergoin[1]. [1]Molec. Virol. Unit, UMR 5087, Univ. Montpellier II, Montpellier, France; [2]Nat. Res. Center, Dokki, Giza, Egypt; [3]Center of Virol, Inst. Rech. Dévelop. (IRD)- Fac. Agric., Cairo Univ., Guiza, Egypt.

9:00 STU **Analysis of a zinc-finger protein from *Choristoneura fumiferana* nucleopolyhedrovirus.** J de Jong[1], B Arif[2], P Krell[1]. [1]Dept. of Microbiology, Univ. of Guelph, Guelph, Ontario, Canada; [2]Canadian Forest Service, Great Lakes Forestry Centre, Sault Ste. Marie, Ontario, Canada.

9:15 **A bacmid of HaSNPV with a 20-kb deletion is still infectious.** Y Huang[1], H Wang[1], X Chen[1], L Yuan[1], JM Vlak[2], Z Hu[1]. [1]Joint-Lab of Invertebr. Virol. and Key Lab. of Molec. Virol., Wuhan Institute of Virology, CAS, Wuhan, P.R. China; [2]Dept. of Virology, Wageningen Univ., Wageningen, the Netherlands.

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

10:00–10:30 **BREAK** Green Mt. atrium

Symposium (Div. of Fungi) Tuesday, 10:30–12:30. Green Mt.–A

Challenges to the use of fungi for control of Acari

Organizer/Moderator: Tove Steenberg.

10:30 **Laboratory and glasshouse evaluation of entomopathogenic fungi against the twospotted spider mite, *Tetranychus urticae*.** D Chandler[1], G Davidson[1], R Jacobson[2]. [1]Horticulture Research International, Wellesbourne, Warwick, UK; [2]Stockbridge Technology Centre, Stockbridge House, Selby, UK.

10:54 **Challenges in using *Neozygites tanajoae* as a classical biological control agent for the cassava green mite in Africa.** I Delalibera Jr.[1], AE Hajek[2], RA Humber[3], FCC Hountondji[4], A Cherry[4]. [1]Dept. of Entomology, Univ. of Wisconsin, Madison WI, USA; [2]Cornell Univ., Ithaca NY, USA; [3]USDA/ARS US Plant, Soil & Nutr. Lab., Ithaca NY, USA; [4]IITA, Cotonou, Rep. of Benin.

11:18 **Novel strategies for control of chicken mites (*Dermanyssus gallinae*) using autodissemination.** T Steenberg. Danish Pest Infestation Lab., Kgs. Lyngby, Denmark.

11:42 **Fungi for control of ticks.** M Samish[1], G Gindin[2], I Glazer[2]. [1]Kimron Veterinary Inst., and [2]Volcani Center, Bet Dagan, Israel.

12:06 **Evaluation of entomopathogenic fungi for control of *Varroa destructor*, an ectoparasite of the honey bee, *Apis mellifera* L.** G Davidson[1], C Birchall[2], J Pell[2], B Ball[2],

K Sunderland[1], D Chandler[1]. [1]Hort. Res. Internat, Wellesbourne, Warwick, UK; [2]Rothamsted Res., Harpenden, UK.

Tuesday, 10:30-12:30. Adirondack Ballroom

POSTERS – 2

Posters should be displayed from Sunday UNTIL NO LATER THAN 1:00 pm, WEDNESDAY

VIRUSES

V-1 **A new densovirus isolated from the african cotton bollworm, *Helicoverpa armigera* Hbn. (Lepidoptera: Noctuidae) in Egypt.** G Fédière[1], M Salah[1], R El-Mergawy[1], M Masri[1], M El-Sheikh[1], A Abd-Alla[2], M Bergoin[2], M El-Far[3], P Tijssen[3]. [1]Centre de Virol., Inst. de Recherche pour le Dévelop., Fac. of Agric., Cairo Univ., Giza, Egypt; [2]Lab. de Pathol. Comparée, USTL, Montpellier, 5, France; [3]INRS-Inst. Armand-Frappier, Laval, Québec, Canada.

V-2 STU **Allotropic determinants of *Galleria mellonella* and *Mythimna loreyi* densoviruses reside on the viral capsid protein.** M El-Far[1], Y Li[1], G Fédière[2], S Abol-Ela[2], P Tijssen[1]. [1]INRS-Institut Armand-Frappier, Laval, Qc, Canada; [2]Center of Virology-IRD, Faculty of Agriculture, Cairo Univ., Egypt

V-3 **Gene organization and content of the *Neodiprion lecontei* NPV genome.** HAM Lauzon[1], C Lucarotti[2], PJ Krell[3], BM Arif[1]. [1]Canadian Forest Service, Great Lakes Forestry Centre, Sault Ste. Marie, Ontario, Canada; [2]Atlantic Forestry Centre, Fredericton, New Brunswick, Canada; [3] Dept. of Microbiology, Univ. of Guelph, Guelph, Ontario, Canada.

V-4 **Structural- functional analysis of the Apoptosis Suppressor Protein P49 from the *Spodoptera littoralis* nucleopolyhedrovirus.** R Galit, N Chejanovsky. Entomol. Dept., Inst. of Plant Protection, ARO,The Volcani Center, Bet Dagan, Israel.

V-5 **Hz-2V genome analysis.** W Kim[1], JP Burand[1,2], CL Afonso[3], GF Kutish[3], Z Lu[3], DL Rock[3]. Depts. of [1]Entomology and [2]Microbiology, Univ. of Massachusetts, Amherst, MA, USA; [3]USDA, 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 Tan[1], JP Burand[1,2]. Depts. of [1]Entomol. and [2]Microbiol., 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 Tan,[1] JP Burand[1,2], W Kim[1], S Nojima[3], W Roelofs[3]. Depts. of [1]Entomology and [2]Microbiology, Univ. of Massachusetts, Amherst, MA, USA; [3]Dept. of Entomology, Cornell Univ., Geneva, NY, USA.

V-8 **The open reading frame 132 of *Helicocoverpa armigera* single nucleopolyhedrovirus is not essential for viral replication *in vitro.*** M Fang, H Wang, X Chen, Z Hu. Joint-Laboratory of Invertebr. Virology and Key Lab. of Mol. Virology, Wuhan Inst. of Virology, Chinese Acad. of Sciences, Wuhan, P.R. China.

V-9 STU **Differential activity of *Helicoverpa armigera* nucleopolyhedrovirus on cotton, chickpea and tomato.** R D`Cunha, PC Stevenson, D Grzywacz. Nat. Resources Inst., Univ. of Greenwich, Chatham Maritime, Kent, UK.

V-10 **Defective baculoviruses increase the pathogenicity of the virus population.** O Simón[1,2], P Caballero[1], T Williams[1], M López-Ferber[2]. [1]Laboratorio de Entomología Agrícola y Patología de Insectos, Depto. de Producción Agraria, Univ. Pública de Navarra, Pamplona, Spain; [2]Génétique de Virus, Laboratoire de Pathologie Comparée, INRA/CNRS/Univ. de Montpellier II. St Christol les Alès, France.

V-11 **Localization and sequence analysis of the *Anticarsia gemmatalis* nucleopolyhedrovirus *25K FP* gene.** ML Souza[1], MEB Castro[1], FR da Silva[1], W Sihler[1], MRS Pedrini[2]. [1]Embrapa Recursos Genéticos e Biotecnologia, Brasília, Brazil; [2]Univ. of Queensland, Brisbane, Australia

V-12 **Baculovirus susceptibility, improved protein production, and resistance to nutrient stress by new *Trichoplusia ni* (BTI Tn5B1-4) High Five™ cell clones.** G-X Li[1,3], Y Hashimoto[2,3], RR Granados[3]. [1]Laiyang Agric. Univ., Laiyang, Shangdong, China; [2]Center for Biosystems Res., UMBI, College Park, MD, USA; [3]Boyce 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 Oers[1], M Doitsidou[1], AAM Thomas[2], JM Vlak[1]. [1]Lab. of Virology, Wageningen Univ., the Netherlands; [2]Dept. of Developm. Biology, Utrecht Univ., the Netherlands.

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

V-15 **Purification and charcaterazation of two viral particles from diseased postlarvae of *Macrobrachium rosenbergii*.** Z Shi[1], D Qian[2], J-R Bonami[3]. [1]Key Laboratory of Molecular Virology, Joint Laboratory of Invert. Virol., Wuhan Inst. of Virol., Chinese Acad. of Sci., Wuhan, China; [2] Zhejiang Inst. of Freshwater Fisheries, Huzhou, China; [3] UMR, DRIM, CNRS/IFREMER/UM2, Montpellier, France.

V-16 **A possible transmission pathway *in vivo* of white spot syndrome virus.** Z Shi, J Zhang, H Wang, Y Xie. Key Lab. of Molec. Virology, Joint Lab. of Invert. Virol., Wuhan Inst. of Virology, Chin. Acad. of Sci., Wuhan, China.

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. Virol., Wuhan Institute of Virology, Chinese Acad. of Sciences, Wuhan, China.

V-18 **Absence of PIF blocks baculovirus ODVs infection after the binding step.** I Kikhno[1], S Gutierrez[1], M Ravallec[1], O Simon[1, 2], P Caballero[2], M Lopez-Ferber[1]. [1] Lab. de Pathol. Comparée, INRA/CNRS/Univ. de Montpellier II. St Christol-Les-Ales, France; [2]Lab. de Entomol. Agrícola 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 Becnel, 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 Levy[1], ÂMF Falleiros[2], F Moscardi[3], EA Gregório[1]. [1]Centro de Microscopia Eletrônica, IBB, UNESP, Botucatu-SP, Brazil; [2]Centro de Ciências Biológicas, UEL, Londrina-PR, Brazil; [3]Centro Nacional de Pesquisa da Soja, Embrapa, Londrina-PR, Brasil.

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

larva midgut cells? SM Levy[1], ÂMF Falleiros[2], F Moscardi[3], EA Gregório[1]. [1]Centro de Microscopia Eletrônica, IBB, UNESP, Campus de Botucatu, Botucatu-SP, Brazil; [2]Centro de Ciências Biológicas, UEL, Londrina-PR, Brazil; [3]Embrapa Soja, Londrina-PR, Brazil.

V-22 **Comparative study on the susceptibility of cutworms (Lepidoptera: Noctuidae) to *Agrotis segetum* NPV and *A. ipsilon* NPV.** S El-Salamouny[1,2,3], M Lange[1], M Jutzi[1], J Huber[3], JA Jehle[1]. [1]State Educ. and Res. Center for Agric., Viticult. and Horticult. (SLFA), Biotechnol/ Crop Prot., Breitenweg, Neustadt/Wstr., Germany; [2]Dept. Econ. Entom. and Pesticides, Fac. of Agric., Cairo Univ., Giza, Egypt; [3]Federal Biol. Res. Centre for Agric. & Forestry (BBA), Instit. for Biol. Control, Darmstadt, Germany.

V-23 **Characterization of a truncated chitinase gene within the genome of the *Cryptophlebia leucotreta* granulovirus.** M Lange, JA Jehle. State Educ. and Res. Center for Agric., Viticult., and Horticult. (SLFA), Biotechnological Crop Protection, Breitenweg, Neustadt/Wstr., Germany.

V-24 **Effects of a protease-expressing recombinant baculovirus on nontarget insect predators of *Heliothis virescens.*** AJ Boughton, JJ Obrycki, BC Bonning. Dept. of Entomol., Iowa State Univ., Ames, IA, USA.

V-25 **Disintegration of the peritrophic membrane of silkworm (*Bombyx mori*) larvae due to spindles of an entomopoxvirus.** W Mitsuhashi, K Miyamoto. National Inst. of Agrobiological Sciences, Tsukuba, Ibaraki, Japan.

V-26 **Expression of a *Toxoneuron nigriceps* polydnavirus (TnBV) encoded protein, TnBV1, is toxic for lepidopteran insect cells.** R Lapointe[1,2], R Wilson[1], DR O'Reilly[1,3], F Pennacchio[4,5], C Malva[4], JA Olszewski[1]. [1]Dept. of Biol. Sci., Imperial College London, UK; [2]Nat. Resources Canada, Can. Forest Serv., Laurentian Forestry Centre, Sainte-Foy, Québec, Canada; [3]Syngenta, Jealotts Hill Internal. Res. Centre, Bracknell, Berks, UK; [4]Inst. di Genetica e Biofisica-C.N.R., Napoli, Italy; [5]Dipto. di Biologia, Difesa e Biotecnologie Agro-Forestali, Univ. della Basilicata-Macchia Romana, Potenza, Italy.

V-27 **Picorna-like viruses in honey bees: transmission routes and role of *Varroa* mites in infection.** M Shen, N Ostiguy, L Cui, S Camazine, DL Cox-Foster. Dept. of Entomology, The Pennsylvania State Univ., University Park, PA , USA.

V-28 **STU** **DNA polymerase sequence analysis and host range of *Ascovirus* isolates from Indonesia and the United States.** Y.M. Kusumah, GR Carner, Ö Kalkar. Entomology Dept., Clemson Univ., Clemson, SC 29634, USA.

V-29 **STU** **Determination of PhopGV activity by a precise surface-contamination method.** MV Carrera[1,2,3], JL Zeddam[1,2], X Lery[1], A Pollet[1,2], M Lopez-Ferber[3]. [1]IRD, [2]Pontificia Univ. Católica del Ecuador, Quito, Ecuador; [3] Lab. de Pathol. Comp., INRA-CNRS-UMII, Saint-Christol-lez-Alès, France.

BACTERIA

B-1 **STU** **Endospore degradation in an asporogenic, crystalliferous mutant of *Bacillus thuringiensis.*** P Sierra-Martínez[1], JE Ibarra[2], M de la Torre[1], G Olmedo[3]. [1]Depto. de Biotecnol. y Bioingen., Centro de Investigación y de Estudios Avanz. del IPN, México, D.F.; [2]Depto. de Biotecnología y Bioquím., and [3]Depto. de Ingeniería Genética, Centro de Investig. y de Estudios Avanzados del IPN, Irapuato, Gto., México.

B-2 **Destruction of bacterial spores by non-contact ultrasound.** K Hoover[1], N Ostiguy[1], M Bhardwaj[2]. [1]Dept. of Entomology, Penn State Univ., University Park, PA, USA; [2]Ultran Laboratories, Inc., Boalsburg, PA, USA.

B-3 **Laboratory and field experiments for control of *Helicoverpa armigera* based on bitoxibacillin formulation containing *Bt* b–exotoxin *Bt.*** EN Abdullaev. Samarkand State Univ., Samarkand, Uzbekistan.

B-4 **The research and developement 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 **STU** **Environmental distribution, frequency and diversity of *Bacillus thuringiensis* isolates from Spain and Latin America.** CS Hernández[1], A Boets[2], J Van Rie[2], J Ferré[1]. [1]Depto. de Genética, Univ. de Valencia, Burjassot, Spain; [2]Bayer 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 Biotecnol. y Control Biológico, Corporación para Investigaciones Biológicas, Medellín, Colombia.

B-7 ***Aedes aegypti* larval control with *Bacillus thuringiensis* serovar. *israelensis*: long lasting effects of an experimental tablet formulation.** J Hernandez, G Armengol, A Restrepo, S Orduz. Biotechnology and Biological Control Unit. Corp. para Investig. Biológicas, Medellin, Colombia.

B-8 **STU** **Mosquito larvicidity and synergism in transgenic *Anabaena* expressing four genes from *B. thuringiensis* subsp. *israelensis.*** V Khasdan[1], E Ben-Dov[1,3], R Manasherob[1,3], S Boussiba[2,3], A Zaritsky[1,3]. [1]Dept. of Life Sciences, and [2]Microalgal Biotechn.. Lab, Ben-Gurion Univ. of the Negev, Be'er-Sheva, Israel; [3]Bio San Ltd., Ariel, Israel.

B-9 **STU** **Toxicity against larvae of *Aedes aegypti* and synergism with Cry toxins by 3 different Cyt proteins from *B. thuringiensis.*** M Itsko, R Manasherob, E Ben-Dov, N Baranes, V Khasdan, A Zaritsky. Dept. 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 Pootanakit, C Angsuthanasombat, 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 Ernandes[1], K Yamaoka[1], A Oshiro[1], M Umsza Guez[1], VL Del Bianchi[1], I de Oliveira Moraes[2]. [1]Dept. Food Engineering and Technology, UNESP, São José do Rio Preto, Brazil; [2]Univ. of Guarulhos, Guarulhos, Brazil.

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

B-13 **Suitability of genetically modified *Bacillus thuringiensis* WG-001 for safety release on cotton fields.** Z. Shu[1], L Li[1,2], M Sun[1,2], Z Yu[1,2]. [1]Key Lab. of Agric. Microbiol., Ministry of Educ.; [2]Nat. 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-Izquierdo[1,3], G Alvarez-Manilla[1,3], M Pierce[3], M Adang[2,3]. [1]Centro de Investigacion en Alimentacion y Desarrollo, A.C., Hermosillo, Sonora, Mexico; [2]Entomology and [3]Biochemistry & 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.**

JL Jurat-Fuentes[1], LJ Gahan[2], F Gould[3], DG Heckel[4], MJ Adang[1,5]. Depts. of [1]Entomol. and [5]Biochem. & Molec. Biol., Univ. Georgia, Athens, GA, USA; [2]Dept. of Biol. Sciences, Clemson Univ., Clemson, SC, USA; [3]Dept. of Entomology, North Carolina State Univ., Raleigh, NC, USA; [4]Dept. of Genetics, Univ. of Melbourne, Parkville, Australia.

B-16 **Identification of a toxin-binding protein involved in resistance to Cry1Ac in *Heliothis virescens.*** JL Jurat-Fuentes[1], F Gould[2], MJ Adang[1,3]. Depts. of [1]Entomology and [3]Biochemistry and Molec. Biology, Univ. of Georgia, Athens, GA, USA; [2]Dept of Entomology, North Carolina State Univ., Raleigh, NC, USA.

B-17 **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-18 **The chymotrypsin mutans of *Bacillus thuringiensis* Cry1Aa toxins: planar lipid bilayer and light scattering analyses, interaction with *Manduca sexta* midgut receptors.** A Bah[1], K van Frankenhuyzen[2], R Milne[2], R Brousseau[1], L Masson[1]. [1]Biotechnol. Res. Inst., Montreal, Quebec, Canada; [2]Canad. Forest Serv., Great Lakes Forestry Centre, Sault Ste. Marie, Ontario, Canada.

B-19 STU **Proline substitution in α4 affects helical hairpin-flexibility and membrane perturbation of the *Bacillus thuringiensis* Cry4B toxin.** P Ounjai, G Katzenmeier, S Panyim, C Angsuthanasombat. Lab. of Molecular Biophysics, Inst. of Molecular Biology and Genetics, Mahidol Univ., Salaya Campus, Thailand.

B-20 STU **Characterization of the cloned Cry4B domain III fragment.** P Chayaratanasin[1], C Pothiratana[1], G Katzenmeier[1], S Panyim[1], D Gerber[2], Y Shai[2], C Angsuthanasombat[1]. [1]Laboratory of Mol. Biophysics, Inst. of Molecular Biol. and Genetics, Mahidol Univ., Salaya Campus, Nakornpathom, Thailand; [2]Dept. of Biological Chemistry, Weizmann Inst. of Science, Rehovot, Israel.

B-21 **Mobility of plasmid-borne genes encoding disease of a New Zealand scarab pest, *Costelytra zealandica.*** M O'Callaghan, SJ Dodd, MRH. Hurst, CW Ronson[1], TA Jackson, T.R. Glare. Biocontrol and Biosecurity Group, AgResearch, Lincoln, New Zealand; [1]Dept. of Microbiol., Univ. of Otago, Dunedin, New Zealand.

B-22 **Maximizing the use of mass spectrometry data generated from proteomic analyses of insects with relatively few sequenced proteins.** RJ McNall[1], MJ Adang[1,2]. [1]Biochemistry & Molecular Biology and [2]Entomology, Univ. of Georgia, Athens, GA, USA.

B-23 STU **Analysis of midgut brush border proteins in *Bt* susceptible and resistant *Plutella xylostella* larvae using differential two-dimensional electrophoresis.** RJ McNall[1], MJ Adang[1,2]. [1]Biochemistry & Molec. Biology and [2]Entomol., Univ. of Georgia, Athens, GA, USA.

B-24 STU **Interaction of *Bacillus thuringiensis* toxins with *Helicoverpa armigera* midguts.** A Estela, J Ferré, B Escriche. Dept. of Genetics, Faculty of Biology, Univ. of València, Burjassot, València, Spain.

B-25 **Identification of the western spruce budworm midgut receptor for *Bacillus thuringiensis* insecticidal Cry toxins.** AP Valaitis. USDA Forest Service, Northeastern Res. Sta., Delaware, OH, USA.

B-26 ***Wolbachia* in sucking lice.** G Kyei-Poku, DD Colwell, P Coghlin, KD Floate. Lethbridge Research Centre, Agriculture and Agri-Food Canada Lethbridge, Alberta, Canada.

B-27 **Molecular evidence and phylogenetic relationships of *Wolbachia* infection in wasps parasitic on pest flies affecting livestock.** G Kyei-Poku, K Floate, B Benkel, MS Goettel. Lethbridge Res. Centre, Agric. and Agri-Food Canada, Lethbridge, Alberta, Canada.

B-28 STU **Adenylyl cyclase and protein kinase A affected the hemocytes-mediated responses of *Malacosoma disstria* to *Xenorhabdus nematophila* and *Bacillus subtilis.*** V Gulii, CL Brooks, GB Dunphy. Dept. of Natural Resource Sciences, McGill Univ., Montreal, Quebec, Canada.

B-29 STU ***Xenorhabdus* toxins: novel bacterial insecticides.** L Baxter[1,2], A Morgan[1], P Jarrett[1], C Winstanley[2]. [1]Horticulture Res. International, Wellesbourne, Warwick, UK; [2]The Univ. of Liverpool, Liverpool, UK.

B-30 **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-31 **Bacterial male-killers: inherited symbionts with a cut-throat strategy.** MEN Majerus[1], HE Roy[2]. [1]Dept. of Genetics, Univ. of Cambridge, Cambridge, UK; [2]Dept. of Life Sciences, Anglia Polytechnic Univ., Cambridge, UK.

12:30-1:30 **5K FUN RUN / WALK** Lk. Champlain waterfront

EXCURSIONS:

1:00-3:30 **Magic Hat Microbrewery** [box lunch provided]
First bus leaves at 1:00
Second bus leaves at 2:00

4:30-6:00 **Lake Champlain Cruise**
(Be on board the boat by 4:15!)

6:30-9:00 **WATERFRONT BARBEQUE**

WEDNESDAY - 30 July

Symposium (Div. of Mic. Control) Wedn., 8:00-10:00. Green Mt.–A

Microbial control of social insects

Organizer/Moderator: Maureen O'Callaghan.

8:00 **Disease resistance vs. biological control of social insects. And the winner is…** RB Rosengaus. Dept. of Biology, Northeastern Univ., Boston, MA, USA

8:30 **Studies on the resistance mechanisms exhibited by the eastern subterranean termite *Reticulitermes flavipes*.** D Boucias, V Blaeske. Dept. of Entomol. & Nematol., Univ. of Florida, Gainesville, USA.

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.** AC Rath. Valent BioSciences Corp., Asia-Pacific Research Office, Box Hill, NSW, Australia

9:00 **Microorganisms used for control of fire ants.** RM Pereira, DH Oi, DF Williams. USDA-ARS, Center for Medical, Agricultural and Veterinary Entomology, Gainesville, FL, USA.

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.

9:35 **New pathogens and novel strategies for *Vespula* control.** TR Glare[1], AF Reeson[2], AD Austin[3]. [1]AgResearch, Lincoln, New Zealand; [2]Biopesticides, Farming Systems Inst., Qld. Dept. Primary Industr., Indooroopilly, Brisbane, Australia; [3]School of Earth & Envir. Science, and Centre for Evol. Biol. & Biodiversity, Univ. of Adelaide, Australia.

Symposium (Div. of Bacteria) Wednesday, 8:00-10:00. Green Mt.–C

Mode of action of bacterial toxins (Part 1)

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

8:00 **Transgenic Vip crops for insect control.** M Lee, F Walters, H Hart, S Palekar, E Chen. Syngenta Biotechnology, Inc., Research Triangle Park, NC, USA.

8:30 **The ADP-ribosylating mosquitocidal toxin (MTX) from *Bacillus sphaericus* SSII-1.** J Schirmer, I Carpusca, 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 Schwartz[1,2], A Maria Gariria Rivera[3], L Potvin[1], C Berry[3] G Menestrina[4]. [1]Biotechnology Research Inst., Montreal, Que., Canada; [2]GÉPROM and Biocontrol Network, Univ. de Montréal, Que., Canada; [3]Cardiff School of Biosciences, Cardiff Univ., Cardiff, UK; [4]CNR-ITC, Centro di Fisica degli Stati Aggregati, I-38050 Povo, Italy.

9:30 **Biochemical and biophysical properties of PS149B1, a binary toxin from *Bacillus thuringiensis.*** L Masson[1], G Schwab[2], J-L Schwartz[1]. [1]Biotechnology Research Institute, National Research Council, Montreal, Que, Canada; [2]Warwick Consulting Group, Encinitas, CA, USA.

Contributed Papers Wednesday, 8:00-10:00. Lake Champlain

VIRUSES – 4

Moderator: Suzanne Thiem.

8:00 **Use of dsRNA to generate transgenic silkworms resistant to BmNPV.** R Isobe[1],T Matsuyama[1], K Kojima[1,2], T Kanda[2,] T Tamura[2], K Sahara[1], S Asano[1], H Bando[1]. [1]Div. of Applied Bioscience, Grad. School of Agric., Hokkaido Univ., Sapporo, Japan; [2]Insect Gene Engineering Lab., Nat. Inst. of Agrobiol. Sci., Tsukuba, Ibaraki, Japan.

8:15 **Identification and characterization of the inhibitor of apoptosis gene of the entomopoxvirus from *Amsacta moorei* (AmEPV).** Q Li, R Moyer. Dept. of Mol. Genetics and Microbiol., Univ. of Florida College of Medicine, Gainesville, FL, USA.

8:30 ***Pariacoto nodavirus* wild-type virus particles contain a minor protein translated from the second AUG codon of the capsid open reading frame.** KN Johnson, LA Ball. Microbiol. Dept., Univ. of Alabama, Birmingham, AL, USA.

8:45 **Expression and purification of an active superoxide dismutase from *Amsacta moorei* entomopoxvirus (AmEPV).** MN Becker[1], A Bawden[1], D Aramburo[1], W Greenleaf[2], R Moyer[1]. Depts. of [1]Molecular Genetics and Microbiology and [2]Pharmacology, Univ. of Florida, Gainesville, FL, USA.

9:00 **Functional analysis of AcMNPV exon0 (orf141) that codes for a novel RING finger protein.** X Dai[1], T Stewart[2], JA Pathakamuri[2], DA Theilmann[1,2]. [1]Pacific Agri-Food Research Centre, Agric. and Agri-Food Canada, Summerland, BC, Canada; [2]Dept. of Plant Sci., Univ. of British Columbia, Vancouver, Canada.

9:15 **Post-translational modification of AcMNPV GP64 by palmitoylation: mapping and functional studies of GP64 membrane localization.** SX Zhang, Y Han, GW Blissard. Boyce Thompson Inst., Ithaca, NY, USA.

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

9:45 **Functional analysis of the fusion domain of baculovirus F proteins.** M Westenberg[1], O Lung[2], D Zuidema[1], GW Blissard[2], JM Vlak[1]. [1]Lab. of Virology, Wageningen Univ., Wageningen, The Netherlands; [2]Boyce Thompson Institute, Cornell Univ., Ithaca NY, USA.

10:00–10:30 **BREAK** Green Mt. atrium

Wednesday, 10:30-1230. Green Mt. Ballroom

SOCIETY for INVERTEBRATE PATHOLOGY

Annual Business Meeting

Presiding: Harry Kaya.

12:30–2:00 **LUNCH** Adirondack Ballroom

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

Symposium (Cross-Div.) Wednesday, 2:00–4:00. Green Mt.–A

Epizootiological modeling

Organizer/Moderator: David Onstad.

2:00 ***Entomophaga maimaiga* and the Gypsy Moth: Insights from a model.** R.M. Weseloh. Dept. of Entomol., Connecticut Agric. Expt. Station, New Haven, Connecticut, USA.

2:25 **The dynamics of inoculum persistence in the infection of the Colorado potato beetle with *Beauveria bassiana.*** F.A. Drummond and E. Groden. Dept. of Biol. Sciences, Univ. of Maine, Orono, ME, USA.

2:50 **Combining mechanistic and statistical modeling to predict epidemics in insect populations.** G. Dwyer[1], B. Elderd[2], M. Coram[3]. [1]Dept. of Ecology and Evolution, [2]Center for Integrating Statistical and Environ, Sci., and [3]Dept of Statistics, Univ. of Chicago, Chicago, IL, USA.

3:15 **Modeling *Nosema* disease in honey bee colonies.** D.W. Onstad[1], D.W. Crowder[1], Z. Huang[2]. [1]Dept. of Natural Resources and Env. Science, Univ. of Illinois, Urbana, Illinois, USA; [2]Dept. of Entomology, Michigan State Univ., East Lansing, Michigan, 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.

2:00 ***Photorhabdus* and *Xenorhabdus* genes for use in transgenic plants.** T. Hey, S. Bevan, A.Schleper, P. Birkhold, S. Russell, R. Thompson, J. Sheets, Z.S. Li, J. Lira, S. Bintrim, K. Fencil, W. Ni, D. Merlo and T. Meade. Dow AgroSciences, Indianapolis, IN, USA.

2:30 **Toxins from *Xenorhabdus* species.** A. Morgan, M. Sergeant, M. Ousley, L. Baxter, D. Ellis, H. Sirs, S. Lee, P. Jarrett. Hort. Res. Internat., Wellesbourne, Warwick, UK.

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. Abdullah[1], A. White[1], R.J. McNall[2], M.J. Adang[2], D.H. Dean[1]. [1]Dept. of Biochem., The Ohio State Univ., Columbus, OH, USA; [2]Dept. of Entomol., Univ. of Georgia, Athens, GA, USA.

3:30 **Receptors and rafts in Cry toxin action.** M. Zhuang[1,2], R. Xie[1,2], I. Gomez[3], M. Soberón[3], A. Bravo[3], L.S. Ross[2], S.S. Gill[1,2]. [1]Graduate Program in Envir. Toxicology, [2]Dept. of Cell Biology and Neuroscience, Univ. of California, Riverside, CA, USA; [3]Instituto 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.

2:00 **A persistent baculovirus infection in a laboratory-reared culture of *Trichoplusia ni*?** R. Hitchman[1,2], J.P. Burden, L.A. King[2], R.S. Hails[1], R.D. Possee[1]. [1]NERC Institute of Virology and Envir. Microbiology, Oxford, UK; [2]School of Biol. and Molec. Sci., Oxford Brookes Univ., Headington, Oxford, UK.

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.

2:30 **Polydnavirus integration in gypsy moth cells.** D.E. Gundersen-Rindal, D.E. Lynn. U.S. Dept. of Agriculture, Insect Biocontrol Lab., Beltsville, MD, USA.

2:45 **A phage-displayed peptide can inhibit infection of white spot syndrome virus of shrimp.** G. Yi, Y. Qi, J. Qian, Z. Wang. College of Life Sciences, Wuhan Univ., Wuhan, Hubei, P.R.China.

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

3:15 **Baculovirus diversity: Establishment of a natural classification system using molecular phylogeny.** M. Lange, H. Wang, J.A. Jehle. State Education & Research Center for Agric., Viticulture & Horticulture (SLFA), Biotechnological Crop Protection, Breitenweg, Neustadt/Wstr., Germany.

3:30 **BUGs: The Baculovirus Updated Genome site enabling *virologists* to keep pace with genome sequencing.** S.L. Turner, M. Thurston, R.D. Possee, D. Field. NERC Inst. of Virol. and Envir. Microbiology, Oxford, UK.

4:00–4:30 **BREAK** Green Mt. atrium

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

Baculovirus genomics

Organizers/Moderators: James Maruniak, David Theilman.

4:30 **Genomics and evolution of the *Neodiprion lecontei* "nucleopolyhedrovirus".** B. Arif[4], H.A.M. Lauzon[1], C. Lucarotti[2], P. Krell[3]. [1]Great Lakes Forestry Centre., Sault Ste. Marie, ON. Canada; [2]Canad. Forstry Serv., Fredericton, NB, Canada; [3]Dept. of Microbiol, Univ. of Guelph, ON, Canada.

4:54 **Sequence analysis of the genome of *Neodiprion sertifer* single-nucleocapsid nucleopolyhedrovirus.** J.E. Maruniak, A. Garcia-Maruniak, A. Doumbouya, T. Merritt, J.-C. Liu, J. Lanoie, R. Kesari. Dept. of Entomology & Nematology, Univ. of Florida, Gainesville, Florida, 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

5:42 **Influence of hosts on the diversity of the *Baculoviridae.*** E.A. Herniou[1,2], J. Olzewski[1], D. O'Reilly[3], J. Cory[2]. [1]Dept of Biological Sciences, Imperial College London, London, UK; [2]NERC CEH-Oxford, Oxford UK; [3]Syngenta, Bracknell, UK.

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).** L.G. Willis[1], T. Stewart[2], R. Seipp[1], Martin Erlandson[3,4], D.A. Theilmann[1,2]. [1]Pacific Agri-Food Res. Centre, Agric. Agri-Food Canada, Summerland, BC, Canada; [2] Agric. Sci., Univ. of British Columbia; [3]Saskatoon Res. Centre, Agric. Agri-Food Canada, Saskatoon, SK, Canada; [4]Dept. of Applied Microbiol., Univ. Saskatchewan, Canada.

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

Mode of action of three-domain Cry toxin family (Part 2)

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

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

5:10 ***Bacillus thuringiensis* Cry1 toxin activity: role of domain I components and modulation by the physico-chemical environment.** V. Vachon[1,2], R. Laprade[1,2], J.-L. Schwartz[1,2,3], L. Masson[2,3]. [1]Groupe d'étude des protéines membranaires, Univ. de Montréal, Montreal, Quebec, Canada; [2]Biocontrol Network, and [3]Biotechnol. Res. Inst., National Research Council, Montreal, Quebec, Canada

5:50 **Functional properties of *Bacillus thuringiensis* toxin receptors in a *Drosophila* S2 cell system.** M.J. Adang[1,2], J.L. Jurat-Fuentes[1], G. Hua[1]. Depts. of [1]Entomol. and [2]Biochem. & Molec. Biol., Univ. Georgia, Athens, GA, USA.

WEDNESDAY PM

Contributed Papers Wednesday, 4:30-6:30. Lake Champlain

FUNGI – 3

Moderator: Melanie Filotas.

4:30 **Phylogeography of the insect pathogenic fungus, *Metarhizium*.** M.J. Bidochka, C.L. Small. Dept. of Biol. Sci., Brock Univ., St. Catharines, Ontario, Caanda.

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. Castrillo[1], E. Groden[2], S.L. Annis[2], J.D. Vandenberg[3]. [1]Dept. of Entomol., Cornell Univ., Ithaca, NY, USA; [2]Dept. of Biol. Sciences, Univ. of Maine, Orono, ME, USA; [3]USDA-ARS, US Plant, Soil & Nutrition Lab., Ithaca, NY, USA.

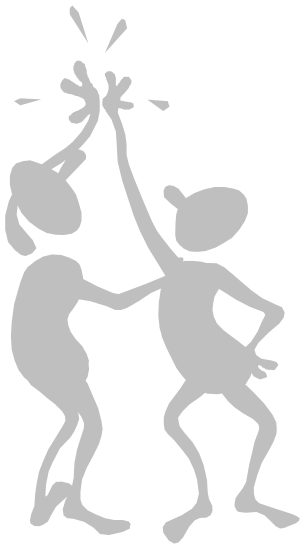
5:45 **Evaluation of entomopathogenic fungi for microbial control of the greenhouse pests *Myzus persicae* and *Aphis gossypii.*** M Filotas[1], S Wraight[2], J Sanderson[1]. [1]Dept. of Entomology, Cornell Univ., Ithaca, NY, USA; [2]USDA Agriculture Research Service, US Plant, Soil, & Nutrition Lab.,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

6:00 **The effects of drying on germination and activity of *Metarhizium anisopliae* var. *acridum* conidiospores.** B.P. Magalhães[1,2], D.G. Boucias[1]. [1]Entomol. & Nematol. Dept., Univ. of Florida, Gainesville, Florida, USA; [2]Permanent 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. Wright[1], M.A. Jackson[2], W.J. Connick[1]. [1]USDA, Agric. Research Service, Southern Regional Research Center, New Orleans, LA, USA; [2]USDA, Agric. Research Service, National Center for Agric. Utilization Research, Peoria, IL, USA.

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

AWARDS CEREMONY



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ABSTRACTS 2003

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STU indicates papers being judged for graduate student presentation awards

SUNDAY - 28 July

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

Pathogen-midgut interactions

Symposium. Sunday, 10:30

Mosquito midgut as a physical and biological barrier to malaria transmission

Mohammed Shahabuddin

Lab. of Malaria and Vector Research, National Institute of Allergy nnd Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20892, USA

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 slender, motile ookinetes. Studies with the avian parasite *Plasmodium gallinaceum* showed the ookinetes recognize the intestinal wall of *Aedes aegypti* via a carbohydrate ligand-receptor interaction. The bound parasite then moves about on the midgut lumenal surface before invading an epithelial cell. Unlike the ookinete of the rodent 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.

Symposium. Sunday, 11:00

Arbovirus-vector interactions in midguts of the mosquito, *Aedes aegypti*

Ken E. Olson, Kristine E. Bennett, Irma Sanchez-Vargas, Carolina Barillas-Mury, Carol D. Blair, William C. Black IV, and Barry J. Beaty

Arthropod borne and Infectious Diseases Laboratory, Foothills Research Campus, Dept. of Microbiology, Immunology, and Pathology, Colorado State Univ., Fort Collins, CO 80523, USA

Mosquitoes acquire arthropod-borne viruses (arboviruses) by ingesting an infected blood meal. Soon after ingestion, virus enters the midgut epithelial cells, replicates, escapes, and then disseminates to secondary target organs. Virus infects salivary glands, replicates, sheds into the saliva and is transmitted during a subsequent bite. If an arbovirus is blocked at early stages of midgut infection, the mosquito has a midgut infection barrier (MIB). MIB is most likely controlled by vector genes involved with virus processing, attachment, penetration, and uncoating. In *Aedes aegypti*, a region on chromosome 2 and another on chromosome 3 have been associated with MIB to dengue-2 (DV2; Flaviviridae) virus infection using quantitative trait loci (QTL) mapping. If an arbovirus readily infects the midgut but is unable to escape and disseminate to other organs, the mosquito has a midgut escape barrier (MEB). A region of *Ae. aegypti* chromosome 1 has been associated with MEB of DV2 by QTL mapping. What is the nature of the genes that control MIB and MEB? We have shown that genes controlling proteolytic processing in the gut lumen may be important for efficient virus infection. For instance, the two MIB QTLs on chromosome 2 and 3 contain early and late trypsin genes, respectively. In addition, we have observed that trypsin inhibitors significantly reduce vector competence for DV2. We are also characterizing genes in the RNA interference (RNAi) pathway of *Ae. aegypti* and other mosquito vectors that could play an essential role in modulating permissiveness of midgut cells for arbovirus infection and that may be critical for expression of the MEB. It is now clear that mosquito cells possess the RNAi machinery since it can be activated by introducing double stranded RNA. Can arboviruses also activate RNAi since most arboviruses form dsRNA during replication? Could genes controlling RNAi be involved in vector competence? In plants, it has been shown that mutations of components of the RNAi pathway can lead to enhanced pathogenesis of RNA virus infections and that many plant viruses and at least one insect virus have evolved RNAi suppressor genes. These observations open the door for investigations of critical virus-vector interactions that may play important roles in identifying genes involved with MIB, MEB, and vector competence.

Symposium. Sunday, 11:30

The complex relationship between a simple RNA virus and its heliothine insect host

Terry Hanzlik and Karl Gordon

CSIRO Entomology, Box 1700, Canberra, ACT 2601 Australia

As a member of the *Tetraviridae*, the *Helicoverpa armigera Stunt Virus* (HaSV) is a very simple virus. It has only three genes encoded on two message sense RNA strands that are encased in a capsid composed of 240 copies of the same protein. The virus is also simple in its selection of tissues and hosts to infect: only midguts of heliothine caterpillars. Yet study of the relationship between this simple virus and larval *H. armigera* discloses a complex, almost paradoxical pathobiology. The virus appears to be relatively common in *H. zea* populations of US cotton fields but never has been reported as causing disease in this well studied population. The virus is both horizontally and vertically transmitted, yet the latter phenomenon occurs in spite of an acute virulence by the virus towards host neonate larvae at minute, (1000 particle) doses. The virulence of HaSV leads to rapid stunting and death and is associated with a reduction in the number of midgut cells. The reduction is caused by an increased rate of cell rejection combined with a reduced rate of cell regeneration. In an extreme example of developmental resistance, the susceptibility seen in early larval development turns into total immunity during later larval development, even towards chronic, maximal doses. HaSV infections establish themselves in foci of associated cells in midguts of host insects from all stages of larval development. Yet the infections of later instar larvae disappear while those established earlier in development expand to encompass the whole midgut. Despite its eventual infection of the whole midgut, the initial infection of HaSV occurs only in a limited region of the anterior midgut as established by reporter gene activity from engineered "one-way" viruses and virus-like particles unable to produce infective progeny. Moreover, the virus infects all major midgut cell types yet appears to have its receptor only on a subset of goblet cells. These and other apparent paradoxes can be explained by reasonably simple hypotheses. Finally, HaSV and like viruses have unique structural properties that can be exploited for three novel technologies of use to agriculture and medicine.

Symposium. Sunday, 12:00

Midgut barriers to baculovirus infection

Jan O. Washburn and Loy E. Volkman

Dept. of Plant and Microbial Biology, Univ. of California-Berkeley, Berkeley, California, 94720-3102, USA

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 epidermal 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, 3) and 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

The role of RNA interference in arbovirus infections of mosquitoes

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

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 20-25 nucleotides in length with sequence homology to the dsRNA trigger, can be demonstrated in resistant 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.

Symposium. Sunday, 2:24

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.

Symposium. Sunday, 2:48

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

Ruud A. de Maagd[1], Petra L. Bakker[1], Tsanko Gechev[1], Tjie-Yien Man[1], Salvador Herrero[1], and William J. Moar[2]

[1]Plant Research International, Wageningen, The Netherlands; [2]Dept. 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. Adang[1,2] and Rebecca J. McNall[1]

[1]Biochemistry & Molecular Biology and
[2]Entomology, 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-LeRoux[1,2], Christophe Buisson[1], Patricia Nel[1], Myriam Hajaij[1,] Sinda Fedhila[1], Elisabeth Guillemet[1], Laurence Fiette[3], and Didier Lereclus[1,2]

Unité Génétique Mirobienne et Environnement, INRA, la Minière, 78285 Gouyancourt, Cedex France[1], Unité de Biochimie Microbienne[2] and Unité d'Histotechnologie et Pathologie[3,] Institut Pasteur, 75724 Paris, France

The main insecticidal activity of *Bacillus thuringiensis (B*t) is due to the larval ingestion of the insect specific Cry toxins. However, both *Bt* crystal minus 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, entero toxins, 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 septicemia. 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* gen*e* (green fluorescence protein*)* and the promotors 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]Salamitou S.** *et al.,* 2000 The regulon PlcR is involved in the opportunistic properties of *Bacillus thuringiensis* and *Bacillus cereus* in mice and insects. *Microbiology* **146** :2825-283. **[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:** 3296-3304. **[3] Fedhila, S., Gohar, M., Slamti, L., Nel, P., Lereclus,D.,** 2003. The *Bacillus thuringiensis* PlcR-regulated gene *inhA2* is necessary, but not sufficient, for virulence. *J. Bacteriol.* In press.

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

Conservation microbial biocontrol

Symposium. Sunday, 2:00

Conservation of *Neozygites fresenii* in cotton

Donald Steinkraus

Dept. of Entomology, 319 AGRI, Univ. of Arkansas, Fayetteville, AR 72701, USA

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 caterpillar, 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.

Symposium. Sunday, 2:30

Managed field margins as refugia for *Pandora neoaphidis*

P.A. Shah and J.K. Pell

Plant and Invertebrate Ecology Division, Rothamsted Research, Harpenden, Hertfordshire, AL5 2JQ, UK

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 been higher in the field margin than the crop. Species-specific primers can discriminate between *P. neoaphidis* and other Entomophthorales, but we are currently unable to track *P. neoaphidis* isolates obtained from field collected aphids.

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, 3:00

Hedgerows, flies, aphids and winter survival of Entomophthorales

Jørgen Eilenberg and Charlotte Nielsen

Dept. of Ecology, The Royal Veterinary and Agricultural Univ., Thorvaldsensvej 40, DK 1871 Frb C., Denmark

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 schizophorae* and *Strongwellsea* spp.
2) Aphids (*Sitobion avenae*) – *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, 3:30

Conservation of natural enemies of weeds and plant pathogens

Harry C. Evans

CABI Bioscience, UK Centre (Ascot), Silwood Park, Ascot, Berkshire, SL5 7TA, UK

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 form 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 those natural ecosystems which are sources of potential biocontrol agents.

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

MICROSPORIDIA

Contributed paper. Sunday, 2:00

Protein fingerprinting microsporidian isolates from European populations of *Lymantria dispar*

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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 harvested 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.

Contributed paper. Sunday, 2:15

Is permissiveness of *Lymantria dispar* larvae to microsporidian infections determined by the host's immune response?

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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 lead to a slight, temporary decrease in hemocyte numbers. In a second step we employed 'pseudoparasitization' by gamma-irradiated braconid parasitoids 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

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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* L. - a forest pest insect

Dörte Goertz[1], David Onstad[2], David Crowder[2], Andreas Linde[1]

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Microsporidia not know in North American Gypsy Moth, *Lymantria dispar* L., 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 difference equations, 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 und the growth of the host population.

ANDERSON, R. M. & MAY, R. M. (1981): The population dynamics of microparasites and their invertebrate hosts. - Philosophical transaction of the Royal Society of London, Series B: Biological Sciences 291: 451 - 524. DWYER, G.; DUSHOFF, J.; ELKINGTON, J. S. & LEVIN, S. A. (2000): Pathogen-Driven Outbreaks in Forest Defoliators Revisited: Building Models from Experimental Data. - The American Naturalist 156: 105-120

Contributed paper. Sunday, 3:00

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

Nguya K. Maniania[1], Larry J. Vaughan[2], Ellie O. Osir[1] and Elizabeth O. Ouna[1]

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The microsporidium *Johenrea 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 (10^6, 10^7 and 10^8 spores ml^{-1}). Mortality varied between 80 and 100% 32 days after treatment. There were no significant differences in mortality between concentrations in both species. The LT_{50} 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 10^4, 10^5, or 10^6 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, 10^7 and 10^8 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.

SYMPOSIUM (Div. of Microsporidia). Sunday, 4:20-6:20

Evolutionary strategies and adaptations for survival among microsporidian parasites in aquatic ecosystems

Symposium. Sunday, 4:20

Vertical transmission and sex ratio distortion in the Microsporidia

Alison M. Dunn

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The Microsporidia frequently make use of vertical transmission during their complex life cycles, yet the importance of this transmission route and its impact on the parasite-host relationship have never been specifically investigated. We surveyed the diversity and distribution of vertically transmitted microsporidia, and tested for host sex ratio distortion. We found that vertically transmitted microsporidia were widespread amongst freshwater and marine amphipod hosts. We identified at least eleven species of microsporidia including nine novel species. A sex ratio screen revealed a female bias in the distribution of four out of five parasite species tested, while breeding experiments confirmed that two species caused host feminisation. We used sequence data to reconstruct the parasite phylogeny. Mapping of the vertical transmission and sex ratio distortion onto this phylogeny showed that these traits have multiple origins and lead us to propose that vertical transmission may be an ancestral transmission route and that it is associated with host sex ratio distortion. We discuss the role of these parasites in freshwater invasions and their importance for host sex ratio evolution.

Symposium. Sunday, 4:50

The influence of transmission route on the epidemiology of a microsporidian parasite of *Daphnia*

Sandra Lass, Dita B. Vizoso and Dieter Ebert

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The microsporidium *Octosporea bayeri* is a parasite of *Daphnia magna*, a planktonic cyclical parthenogenetic freshwater cladoceran. It is a common pathogen in a *Daphnia* metapopulation system in Southern Finland, where populations are exposed to strong environmental fluctuations including frequent local extinction of host populations. *O. bayeri* reduces lifespan and fecundity of its host. This parasite is directly transmitted, either vertically from infected mothers to their offspring, or horizontally from dead infected hosts to susceptible *Daphnia*. Life-history experiments showed that within-host parasite success is lower when the infections are acquired horizontally. At a population level, however, horizontal transmission seems to be a requisite for parasite persistence. In host populations, in which we did not allow horizontal transmission, infected *Daphnia* were outcompeted rapidly by uninfected hosts. When horizontal transmission was allowed, parasite prevalence increased, suggesting that this mechanism is responsible of the quick increase in parasite prevalence observed in mesocosm experiments and in the field. 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. Kohler[1] and Michael J. Wiley[2]

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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 nigrior* (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 extend 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 (*Rhyacophila*) 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

Theodore G. Andreadis

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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 *Acanthocyclops vernalis* 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 Jun. through Sept., and horizontal transmission via the copepod host from Mar. to May (copepod to mosquito) and Oct. to Dec. (mosquito to copepod). It's survival strategies include: low pathogenicity and high tissue specificity that allow for transstadial transmission of horizontally acquired infections and maximum spore production, reliance on living hosts throughout most of its life cycle with over wintering in the copepod, 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. *Hyalinocysta chapmani,* a parasite of *Culiseta melanura* and *Orthocyclops modestus* is found in a comparatively stable, forested, subterranean habitat is inundated with water throughout the year. Copepods are omnipresent and *Cs. melanura* has overlapping broods. *H. chapmani* is maintained in a continuous cycle of horizontal transmission between each host from Jun. through Nov. but lacks a developmental sequence leading to transovarial transmission in the mosquito host. It similarly relies on living hosts for most of its life cycle and over winters in diapausing mosquito larvae and copepods. Transstadial transmission does not occur and there is no polymorphic development in the mosquito host. The spatial and temporal overlap of both mosquito and copepod 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.

CONTRIUTED PAPERS. Sunday, 4:20-6:20.

VIRUSES – 1

Contributed paper. Sunday, 4:20

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

Martin Erlandson[1], Sarah Newhouse[1], Alida Janmaat[2], Keith Moore[1], Judith Myers[2], and David Theilmann[3]

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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 resistence to Bt in cabbage looper 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 LC_{50} (IU/ml of diet) in laboratory assays of F1 larvae. All greenhouse populations were significantly less susceptible to Bt than a laboratory culture. Baculovirus infection rates ranged as high as 12% in surveyed populations in some greenhouses. Preliminary microscopic analysis indicated that both single nucleopolyhedrovirus (SNPV) and multiple nucleopolyhedrovirus (MNPV) baculoviruses occurred and species-specific PCR analysis demonstrated that *Autographa californica* MNPV (AcMNPV)- and *Trichoplusia ni* SNPV (TnSNPV)-like isolates were present in *T. ni* populations. The TnSNPV isolates were the most prevalent, representing 49 of the 57 isolates collected in the eight greenhouses sampled over the course of the season. Restriction 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 (TnSNPV-RJ) previously collected from cabbage looper populations from New York state (R. Jaques, 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 TnSNPV-RJ isolates. There was no significant differences in LD_{50} values for TnSNPV and AcMNPV-like isolates in 2nd instars (LD_{50}s ranging from 5 to 10 PIB/larva) or 4th instars (LD_{50}s ranging from 10 to 20 PIB/larva). However, LD_{50}s 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.

STU Contributed 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* (*Spe*xNPV) 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 vivo 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.

STU Contributed paper. Sunday, 4:50

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

X. Sun[1, 2, 3], H. Wang[1], X. Sun[1], X. Chen[1], W. van der Werf[2], J.M. Vlak[3] and Z. Hu[1]

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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 ecdysteroid 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 HaSNPVs 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

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 AaIT gene, provided significantly better protection of cotton bolls against bollworm damage than wild-type HaSNPV or HaSNPV *egt*-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 (101-06-10-01, 2001AA214031 and 2001AA212301), NSFC projects (30025003 and 39980001) and a joined grant from the Chinese Academy of Sciences and The Royal Netherlands Academy of Sciences (01CDP023).*

Contributed paper. Sunday, 5:05

Sunshine and infection by NPV in field populations of western tent caterpillars

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For field populations of Lepidoptera, variation in environmental conditions are likely to influence their growth, development and interactions with natural enemies including diseases. One weather variable that may have an impact on infection by nucleopolyhedrovirus of forest caterpillars is hours of sunshine during the period of larval growth. Several hypotheses of how sunshine and viral infection might interact are that: (1) increased sun exposure could destroy virus and reduce infection, (2) with more hours of sunshine infected caterpillars could increase their body temperatures through basking and thus develop a "behavioural fever" detrimental to infection, or (3) increased temperatures associated with opportunities to bask in the sun could speed growth, infection rate and movement of larvae and increase the production and distribution of occlusion bodies and initiate an epizootic. To determine if sunshine and viral infection are related, we looked for an association between hours of sunshine in May and levels of infection in populations of western tent caterpillars in southwestern British Columbia. This relationship was significant with higher levels of infection occurring in years with more hours of sunshine. To test if variation in sun exposure modified growth, development, movement and transmission of NPV, we carried out a field experiment in which the exposure to the sun was modified among family groups to which 8 larvae infected with NPV were added. We observed that family groups of tent caterpillars with greater sun exposure grew and developed faster and built significantly more tents (moved more) than those in more shaded locations. Levels of infection in the two treatment groups were the same 8 and 17 days after the addition of infected larvae to the families. However, for families that had infected individuals 8 days after contamination, twice as many of the sun-exposed families compared to shaded families were also infected 17 days later (experienced a second disease cycle). Because of small sample sizes this difference was not statistically significant although it indicates a trend for more infection. Incorporation of a variable mimicking the variation in hours of sunshine into a disease dynamics model showed that this increased the parameter space over which cyclic population dynamics occurred. Thus two hypotheses – UV breakdown of virus and behavioural fever with increased sun exposure could be rejected while increased production of virus in western tent caterpillars in sunny springs is supported.

Contributed paper. Sunday, 5:20

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

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False codling moth, *Cryptophlebia leucotreta* (Meyrick) (Lepidoptera: Olethreutidae), is a fruit pest of citrus, macadamias, stone fruits, avocadoes and litchis, in southern Africa. Chemical control of *C. leucotreta* is problematic for a number of reasons. Recently, a novel isolate of the *Cryptophlebia leucotreta* granulovirus (CrleGV-SA) was described by restriction endonuclease analysis. In surface dose bioassays on artificial diet, LC_{50} and LC_{90} values with neonate larvae were estimated to be 4.095×10^3 OBs/ml and 1.185×10^5 OBs/ml respectively. LT_{50} and LT_{90} 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 1.08×10^7 to 3.819×10^{10} OBs/ml. In surface dose bioassays with fifth instar larvae LC_{50} and LC_{90} values were estimated to be 2.678×10^7 OBs/ml and 9.118×10^9 OBs/ml respectively. LT_{50} and LT_{90} 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* larval 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.

Contributed paper. Sunday, 5:35

Advances towards improving the insecticidal properties of AgMNPV

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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 low speed of kill of wt AgMNPV, which becomes extremely important in areas with lower temperatures (20ºC). 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 *IPpo*I 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 ecdysone, 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 LT_{50} across virus concentrations: 2.2 days) and exhibited a higher virulence (LC_{50} 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 traceabilty 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'Amico[1], John Podgwaite[1], Ralph Webb[2], Kevin Thorpe[2], Roger Fuester[3], Mike Valenti[4], Randy Pfeiffer[5], Phil Taylor[3], and Jim Slavicek[6]

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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 latter 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.

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

BACTERIA – 1

Contributed paper. 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.

STU Contributed paper. Sunday,4:35

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

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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.*

STU Contributed paper. Sunday, 4:50

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

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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 proteinases. Resistance mechanisms in a Dipel-selected European corn borer (ECB) strain were studied by comparing Cry toxin binding to BBM vesicles and larval gut proteinase 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 Cy1Ac 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 proteinases in resistant ECB larvae was reduced 56%, and Cry1Ab protoxin activation by proteinase extracts from the resistant larvae 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 or even semi-truncated toxin at low to moderate levels may increase the potential of resistance development in target pests.

STU Contributed paper. Sunday, 5:05

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

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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

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

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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

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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 Cry11A + Cyt1Aa (3:1) delayed the onset of resistance and ultimately conferred a lower level of resistance than selection with Cry11A 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

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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

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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 insidiosis*), lacewings (*Chrysopa carnea*), praying mantids (*Tenodera aridifolia sinensis*), and parasitic wasps (*Anaphes ioles*). 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

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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

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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 approach for greenhouses. Trials 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-vector technology not only contributes to pest management but also improves fruit yield and quality by improving pollination and fruit set.

STU 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 impatiens 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/10,000 square feet). Applications were made weekly for three weeks. Samples of pollen bearing impatiens 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/m^2, applied once). NemasysF, Mycotal and water treatments were applied weekly in 8 plots (2.5 m^2 each) with and 8 plots without predatory mites, with a total of 9 applications.

After 5 weekly applications of NemasysF (10^5 nematodes/m^2 + 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 (10^7 conidia/m^2 with Addit) 38% less thrips were found compared to plots with weekly water applications. At harvest, after 9 weekly applications of

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 NemasysF 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

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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 pests, while application of broad-spectrum insecticides threatens the IPM strategy by destabilising 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 Mycotrol®. Comparison of oil and emulsifiable formulations suggest 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.

Contributed paper. Sunday, 5:50

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

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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 18cm 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 10^6 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.

Contributed paper. Sunday, 6:05

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

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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 topically sprayed and maintained on the treated substrate for 24 h at 100% relative humidity, 6-day (25ºC) median lethal rates (LR_{50}s) of this isolate against CEW, BAW, DBM, FAW, ICW, ECB, CL, and BCW were 4, 5, 7, 11, 12, 98, 125, and 273 conidia/mm^2, respectively. The respective LR_{50}s of commercial strain GHA against these pest species were 9, 67, 97, 1213, 29, 1668, 541, and 3504 conidia/mm^2. Use of LR_{50} versus median lethal dose ratios (comparing LR_{50}s of each isolate to a "standard" strain) generated similar rankings of isolate virulence.

MONDAY - 28 July

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

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

J. Langewald[1], N. E. Jenkins[2], B. Ali[3], M. Brüntrup[4] and D. Moore[2]

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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 tonnes 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. Whilst 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 developed 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 biopesticdes 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 Grzywacz[1], Uthai Ketunuti[2] and Hilary Warburton[1]

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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 focussed 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 be 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 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 three years for generation 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.

Symposium. Monday, 9:37

"Evolutionary ecology" of the microbial pesticide industry: Does size really matter?

Michael B. Dimock

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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

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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 apoptotis in 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 Gray[1], Frederick Gildow[2], Diana Cox-Foster[3], Marina Caillaud[4]

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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 hemocoel. 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 the identification 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

Apoptosis as a defense response against virus infection in insects

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

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Apoptosis 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 *Hz*SNPV in plasma of *Heliothis virescens*

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

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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 *Ac*MNPV infective foci in tracheoblast cells of *H. virescens* and *M. sexta* have been reported, and these processes were inhibited by prior polydnavirus infection or parasitization. Phenoloxidase 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 *Helicoverpa 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 ($7.7 \pm 3.3 \times 10^5$ to $1.8 \pm 1.3 \times 10^4$). 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)* become 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 intrahemocoelically 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^os) 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^os and 4^{48}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^{48}-stage insects; whereas, titers increased in 4^0-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 diethydithio-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^o- and 33% of 4^{48}-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.

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

BACTERIA – 2

Contributed paper. Monday, 8:00

Enduring toxicity of transgenic *Anabaena* expressing mosquito larvicidal genes from *Bacillus thuringiensis* subsp. *israelensis*

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Solusi Univ., PO Solusi, Bulawayo, Zimbabwe

Persistence of biological control agents against mosquito larvae was tested under simulated field conditions. Mosquito larvicidal activity of transgenic *Anabaena* PCC 7120 expressing *cry4Aa*, *cry11Aa* and *p20* from *B. thuringiensis* subsp. *israelensis* (*Bti*) was compared with *Bti* itself (as Bactimos 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. napus*/Bt-*B. rapa*: Who will win?

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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_1 hybrids and BC_1 progeny showing the presence of the GFP-Bt transgenes were backcrossed

with the appropriate *B. rapa* parent to produce BC_1 and BC_2 respectively.

Laboratory colonies of local diamondback moth (DBM) populations in eastern and western Canada were established, and bioassays performed to determine the LD_{50} and LD_{95} values of Safer's BTK™ 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* F_1 hybrids, BC_1 and BC_2 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* (BC_2 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

Leah S. Bauer[1,2] and Deborah L. Miller[1]

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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^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 bioinsectides. Novodor®, formulated with *Bt* var. *tenebrionis* and its Cry3Aa1 toxin, had no effect on EAB adults. However, Raven®, 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)

Italo Delalibera Jr.[1], Jo Handelsman[2] and Kenneth Raffa[1]

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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 yanoikuyae*.

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

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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 efficiency of different strains of *Bt subsp. thiringiensis* 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.

Contributed paper. Monday, 9:30

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.

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

Diseases and pathobiology of aquatic invertebrates

Symposium. Monday, 10:30

Quahog Parasite Unknown, an important disease of the hard clam, *Mercenaria mercenaria*

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In 1995, aquaculturists in both Provincetown and Duxbury, MA experienced heavy mortalities of submarket sized hard clams. Upon examination of these clams, a Labyrinthomorphid organism (named QPX) was found to be the cause of the disease. Since that time, QPX has been identified in several populations on Cape Cod, MA; on the Atlantic coast of Virginia; in New Jersey and recently in Raritan Bay in New York.

Studies by this investigator have shown QPX is first found in the mantle and gills indicating the disease enters the tissues from the water column. The inflammatory response is often intense and results in ineffective granuloma formation (and visible nodules and swelling in the mantle) around proliferating parasites. The parasite forms identified both in tissue section and in culture (sporangia containing endospores and thalli) produce thick mucus. Histologically heavy mucus production appears to provide a pathogenic mechanism by inhibiting phagocytosis by the clams hemocytes.

Evaluation of cell culture conditions show that QPX grows best at a pH > 8.0, salinities of 30 ppt and temperatures of 24E C but dies at temperatures of 32E C. These findings correlate with environmental data from the field. Field studies and anecdotal information in MA suggested that disease was more severe in animals that originated from more southern location, but was not conclusive. More recent work by other researchers have shown this is probable.

Both field and laboratory study, accomplished by exposing naive clams to infected clams, has demonstrated that the agent is directly infective and does result in significant mortality in the laboratory as well as field settings.

Symposium. Monday, 10:50

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

Nancy A. Stokes[1], Lisa M. Ragone Calvo[1], Kimberly S. Reece[2] and Eugene M. Burreson[1]

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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 Labyrinthulomycota and for QPX specifically. These tools targeted different regions of the small subunit ribosomal RNA (SSU rRNA) gene. The general labyrinthulomycete 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 DNA 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 – aberrent 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 mollusc 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 Ross[1], Emmanuel Egbosimba[1], Nicole Brun[1,2], Monica Bricelj[1], Thomas MacRae[1,2], Joanne Harding[3], Cyr Couturier[3], and Jay Parsons[3]

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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* acclimatized at 5ºC and subjected to 10ºC 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 (10ºC increase for 3h), and in bay scallops and hard clams (*Mercenaria mercenaria*) following acute cold shock (17ºC 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.

Foreign particles collect under an apical/lateral perforated membrane prior to phagocytic uptake. The role played by the fixed phagocytes in the removal of foreign particles

Symposium. Monday, 12:10

Calcinosis: a new disease in lobsters, *Homarus americanus*

Alistair D.M. Dove[1], Carl P. LoBue[2] and Paul R. Bowser[3]

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A new disease of lobsters that caused mortalities in wild lobsters during summer 2002 is described as a form of calcinosis. 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. Morbid lobsters were characterized by an orange discolouration of the abdomen, lethargy, an excess of epibionts 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 antennal glands 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 (~23ºC) 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, 10:30-12:15.

NEMATODES

STU Contributed paper. Monday, 10:30

Genomic fingerprinting of *Xenorhabdus* spp. using repetitive sequences and PCR

Heather L. Smith[1], Byron J. Adams[1], Jeff B. Jones[2], and Frank J. Louws[3]

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Entomopathogenic nematodes and their bacterial endosymbionts are important biological control agents against a broad range of soil inhabiting insect species. In the past, much study has been devoted to the insecticidal qualities of the bacterial endosymbionts, yet recently, the diversity, co-evolution, and symbiotic properties of the bacteria are beginning to emerge. For the present study, relationships among 52 strains of *Xenorhabdus* bacteria were analyzed by generating genomic fingerprints based on the amplification of repetitive DNA (BOX element, repetitive extragenic palindromic [REP], and the enterobacterial repetitive intergenic consensus [ERIC]) sequences distributed throughout the chromosome (rep-PCR). The rep-PCR products were analyzed by agarose gel electrophoresis, revealing strain-specific patterns. Analysis of the combined BOX, REP, and ERIC fingerprints showed the formation of 3 distinct clusters that are correlated with the species of nematode from which the bacteria were isolated. However, some strains that were isolated from *Steinernema glaseri* are dispersed paraphyletically throughout the dendrogram, while a few other strains formed unique, independent lineages. This study demonstrated that BOX-, REP-, and ERIC-like DNA sequences are commonly distributed in *Xenorhabdus* strains, and therefore, rep-PCR may provide an efficient and sensitive diagnostic tool for identifying and characterizing the bacterial endosymbionts of entomopathogenic nematodes.

Contributed paper. Monday, 10:45

Evaluation of entomopathogenic nematode strains for the control of *Anoplophora glabripennis*

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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. glaseri*, *S. riobrave* TX, *S. carpocapsae* Sal, and two heterorhabditids; *Heterorhabditis indica* MG-13 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 24ºC. Each bioassay chamber contained a single ALB larva. Bioassays were conducted using third, sixth, and seventh instar ALB larvae. EPNs were applied at 100 IJs / larva. Each treatment had 7 replicates. An additional bioassay was conducted using 10 neonates and 100 IJs 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% mortaliy. 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 Simard[1], Guy Bélair[2] and Julie Dionne[3]

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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 LC_{50} 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 ($2.5x10^9$ 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 m^2 turfgrass enclosure containing 160 *E. orientalis* larvae at rates of 0, 0.4, 1.0, or $2.5x10^9$ *S. scarabaei* per ha. At 31 DAT, every larva recovered in the 3 nematode treatments was *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

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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 (NH_4-N, NO_3-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 NH_4-N, N-NO_3, 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 riobrav_e

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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 nematodes 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*

hypogaea) or two varieties of squash (*Cucurbita pepo*) that either contained ("bitter") or did not contain ("non-bitter") the plant secondary metabolite cucurbitacin.

When compared with unselected nematodes, the Agriotos 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 plant 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

Exploitataion 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 Lepidoteran 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 gyrator* infects *M. brassicae* and other Noctuid pest species. A group of *M. brassicae* larvae known to harbour a persistent baculovirus were parasitised by *M. gyrator* 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 28ºC provided the highest AgMNPV yield. In this conditions, efficiency of virus production was over 75% (with canibalism being the major cause of yield loss). Laboratory production yielded an average of 58.8 hectare 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 Ilia Mechnikov in 1879. Small-scale production continued for about 25 years but appeared not to be very successful. The first bacterial pesticides, Dendrobacillin based on *Bacillus thuringiensis (Bt*) subsp. *dendrolimus (sotto*) and Entobacterin 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*

subspecies including *kurstaki, thuringiensis, tenebrionis, israelensis*. In Russia, small-scale industry provides the production of microbial pesticides based on *Bt*, *Salmonella enteriditis,* (for control rats and mice), *Pseudomonas fluorescens*, and the fungi *Beauveria bassiana, Verticillium lecanii, Trichoderma lignorum,* and others. As to viral insecticides, their production also only occurs in smaller firms. Nowadays, the use of viral formulations in Russia is of more importance in forestry than in agriculture. The production and application of Virin-Diprion®, registered in Russia for European pine sawfly-*Diprion sertifer* control, is considered to be the most reliable. The balance between industrial-scale and small-scale production of microbial pesticides seems to be optimum for future development because both ways have their own merits and demerits.

Contributed paper. Monday, 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?

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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 pupate 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 anispoliae.* 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 these pathogens, together with preliminary findings on their 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.

Contributed paper. Monday, 11:30

Non-infectious disease: A neglected paradigm?

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Non-infectious diseases of humans have become a primary emphasis of human health since the medical revolution brought about by the introduction of antibiotics. Non-infectious diseases of insects, both natural and human induced, are undoubtedly a major influence on insect fitness and survival. Although a large body of entomologically orientated research could rightly be considered within a non-infectious disease paradigm, a search of the literature reveals that few researchers communicate their findings from that perspective. One possible reason is that the current paradigm is not sufficiently developed to facilitate inter- and intra-disciplinary dialogue. For infectious diseases of insects we have a variety of terms, concepts, theoretical constructs and methodologies that not only allow us to exchange ideas but also serve as useful tools for exploring our particular areas of interest, whether it be viruses, fungi, bacteria, nematodes or protozoa. Some of these tools are also useful for investigating non-infectious diseases because both sub-disciplines arise form insect pathology. Non-infectious diseases broadly cover mechanical, chemical and physical injuries and nutritional, genetic and neoplastic diseases. Included within this context are lethal and sub-lethal influences of chemical intoxication and abiotic stress from both natural and anthropogenic sources. A useful parallel can be developed using the survival and fitness of the Colorado potato beetle*, Leptinotarsa decemlineata* exposed to toxins from *Bacillus thuringiensis* and the western flower thrips, *Frankliniella occidentalis* exposed to sub-zero temperatures. In turn, these examples of non-infectious disease can be used to draw analogies with the infectious disease paradigm. As noted by R. Gaugler in his chapter on non-infectious disease, while certain concepts and even terminology are shared, the overlap is not complete. Therefore, the paradigm for non-infectious disease needs to be strengthened in its own right if it is to be useful for unifying this sub-discipline with its own theoretical framework. Such an endeavor is likely to produce a windfall of research tools and theoretical progress as various disciplines, such as pathology, toxicology, physiology and ecology, etc., gain a similar framework for approaching their respective areas of inquiry.

STU Contributed paper. Monday, 11:45

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/spread within the host, and virulence is seen as a measure of the severity of disease.

Contributed paper. Monday, 12:00

Possibility for enhancement of practical pest control based on *Hyphomycetes* fungi

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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 blastospores 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.

Monday, 2:00-4:00.

POSTERS – 1

FUNGI

Poster / Fungi. F-1.

Cordyceps staphylinidaecola, its _Beauveria_ anamorph from Korea, and neotypification of _Beauveria bassiana_

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Studies in Korea of *Cordyceps*, a large genus of ascomycete fungi pathogenic to insects, found specimens of a rare species that also formed a conidial (asexual) state identifiable as the extremely common and important species *Beauveria bassiana*. The *Cordyceps* was best identified as a fungus described from Japan as *C. staphylinidaecola* but also appears to be identical to a fungus from China described as *Cordyceps bassiana* whose conidial state is also *B. bassiana*. *B. bassiana* has been shown to be a series of physically similar but genetically distinct organisms. Taxonomic resolution of the *B. bassiana* species complex depends on fixing the strict use of this species name, and we followed traditional practice by choosing a neotype matching the original host and collection site as closely as possible. The neotype of B. *bassiana* is based on an isolate from *Hyphantria cunea* (fall webworm) collected in Emilia-Romagna, Italy. Molecular studies (being published elsewhere by S. Rehner, ARS, Beltsville) indicate that the Asian isolates of *Cordyceps* with *B. bassiana*-like conidial states differ genetically from the neotype of *B. bassiana* and should not, therefore, be identified as *B. bassiana* in the nomenclaturally strict sense. One of the Korean *Cordyceps*/ *Beauveria* isolates has been induced to produce stromata and (apparently infertile) perithecia in culture. The issues raised by these studies about the biology, taxonomy, and nomenclature of this *B. bassiana* have unusual significance and interest for a wide range of scientists.

STU Poster / Fungi. F-2.

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. However the pathogenicity of HpBa-1 against other scrab 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: Bb876). 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. Futhermore, we researched change of the hemocytes number after each fungal conidia injection. When PMA-7 and Bb876 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 pathogenicty to *H. picea* larva.

Poster / Fungi. F-3.

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 *Paraphaeosphaeria*, *Lasiodiplodia*, *Arthrinium*, *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.

Poster / Fungi. F-4.

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

Leah S. Bauer[1,2], Houping Liu[1], Deborah L. Miller[2], Louela A. Castrillo[3], and John D. Vandenberg[3]

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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* F., 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* LC_{50} and LT_{50} were significantly lower in SPS than in CWB, due in part to the relatively smaller size of SPS 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-5.

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

Magnus Kühne,[1] Stefan Vidal,[2] Kerstin Jung,[3] Dietrich Stephan,[3] and Aziz Lagnaoui[4]

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The Andean Potato Weevil (*Premnotrypes suturicallus*, 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 intensivly 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 6 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 10^8 spores/ml. The weevils were placed with potato leaves below a jute sack (commonly used as shelter trap for the weevils) within 1 m^2 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 1 week. Mortalities due to fungus were 18% (T1) and 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 10^8 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-6.

Genetic recombination among vegetatively compatible strains of *Beauveria bassiana* in a susceptible insect host

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Gene exchange, along with mutation, could alter virulence or host range, and should be considered when assessing the risks of wide scale application of a given mycoinsecticide. Genetic recombination in asexual fungi, including *Beauveria bassiana*, can occur through the parasexual cycle, during which hyphae of vegetatively compatible strains fuse and exchange genetic material. Using nitrate non-utilizing (*nit*) mutants, we assessed vegetative compatibility groups (VCG) among strains of *B. bassiana* representing indigenous strains isolated from diverse insect hosts collected throughout the US and strains that have been mass released, like strain GHA, or field tested for insect control. Genetic similarity among these strains was analyzed using random amplified polymorphic DNA markers. Our data revealed 24 VCGs among the 35 strains tested, with most of these groups comprised of only a single strain. For example, strain GHA, the active ingredient in two mycoinsecticides available in the US, was found to be incompatible with all the other strains tested. We also observed a VCG comprised of nine genetically similar strains isolated from Colorado potato beetles (CPB) from the northeastern part of the US and from Quebec and Ontario, Canada. To determine the likelihood of recombination in the field, VCG studies in vitro were followed by co-inoculation studies of CPB larvae with complementary *nit* mutants of genetically distinguishable strains from the same or from different VCGs. Among the different *nit* pairings tested, heterokaryons were observed in two out of five same-VCG pairs, with only 5 to 15 % of the sporulating cadavers generating a few recombinants. In contrast, none of the infected beetles treated with non-compatible pairs generated recombinants. The large number of VCGs observed and the low frequency of in vivo recombination limited to vegetatively compatible strains indicate that this self/non-self recognition system may be an effective barrier preventing genetic exchange between dissimilar strains in the field.

Poster / Fungi. F-7.

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 beetle. 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 LC_{50}s. Average survival times were computed and compared. 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*.

STU Poster / Fungi. F-8.

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 recessed 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 5cm 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.

STU Poster / Fungi. F-9.

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-muscardine 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.

STU Poster / Fungi. F-10.

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 *Trialeuroides 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 whitefly treated 1, 2, 3 or 4 days prior, or one uninfected whitefly. The incidence of prey feeding indicated by stylet insertion into a whitefly 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 oosporein 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.

Poster / Fungi. F-11.

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 Ugine *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 impact pollen 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.

Poster / Fungi. F-12.

Comparation 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 fungicides 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 ∅). 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 testes 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 44) 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 ($P \leq 0.05$) more conidia than the *B. bassiana* isolates. For the *B. bassiana* isolates, conidial production on wheat was significantly higher ($P \leq 0.05$) for the 1:1 and 1:1.5 volumes than the 1:05 volume. No significant differences were found in conidial production for SPT22 on corn and millet at the volume regimes tested; whereas, for CA 44 there was significantly higher ($P \leq 0.05$) conidial production for the 1:0.5 volume than the other volumes. Conidial production of *M. anisopliae* was significantly higher ($P \leq 0.05$) 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 neoscatellae*, 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.*

Poster / Fungi. F-15.

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 *Otiorhynchus*. 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 entompathogenic fungi may thus provide a potential to minimize the damage caused by *Otiorhynchus* 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.

Poster / Fungi. F-16.

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

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In Danish greenery and Christmas tree plantations two of the most important pests are the nut leaf weevil *Strophosoma melanogrammum* 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 Hyphomycetes 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. melanogrammum* and *M. melolontha*, respectively, under field conditions. A significant control effect of *M. anisopliae* against *S. melanogrammum* was seen, when the fungus was sprayed unto the ground of a Noble fir plantation at 10^{14} 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 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 (Melocont, Agrifutur, Italy) were colonised with *B. 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. melanogrammum* and *M. melolontha*, respectively, in greenery and Christmas tree plantations.

Poster / Fungi. F-17.

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) Cercospora 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 only a narrow choice of chemical insecticides (terbufos, phorate, aldicarb, chlorpyrifos). Many of these chemicals are in jeopardy from resistance or regulatory action. The main fungicides are limited in number and face resistance development. These aspects create an ideal stage for a biologically based, integrated system. Our group is studying the deployment of insect pathogenic fungi to manage the sugarbeet root maggot and wireworm, along with three agents from Montana State University, *Bacillus* sp. LS201, *Bacillus subtilis* MSU127, and *Bacillus mycoides* BAC J, to manage the sugar beet pathogens. These microbial tools are being developed with a view to ultimately integrate them with resistant/ tolerant beet hybrids, microbial control agents of sugarbeet cyst nematode, cultural practices, use of disease and pest predictive models, and induced systemic resistance to create an integrated, biologically based pest and disease management program for sugar beets.

Phase 1 of the entomological component has been the selection of candidate fungi based on the criteria of virulence, conidial production in bench-scale solid substrate fermentation, conidial shelf life, and fungal growth at ecologically relevant temperatures. Of 90 *B. bassiana* isolates from sugarbeet fields, *only two* were efficacious against the larval dipteran in a soil-based bioassay reflecting practical field rates ($2.5x10^5$ – $2.5x10^6$ conidia/gram of a silty clay soil). In contrast, a *M. anisopliae* from Norwegian *Delia antigua*, an isolate from a U.S. soybean cyst nematode, and one from a Canadian *Limonius* sp. were highly effective. (No *M. anisopliae* was isolated from sugarbeet fields using both *Galleria*-baiting and a dodine-based selective agar.) Initial field work with one *M. anisopliae* isolate has indicated considerable sugarbeet root maggot control potential.

Poster / Fungi. F-18.

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.

Poster / Fungi. F-19.

Detection of strains of *Metarhizium* within infected sugar cane borer, *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 *Diatraea 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_9 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.

STU Poster / Fungi. F-20.

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 LD_{50} 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.

Poster / Fungi. F-21.

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

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A total of 91 entomopathogenic fungal samples were collected between June 2001 and March 2002 from localities in Ethiopia that are known to be desert locust recession and/or invasion areas. Fifty-six samples were identified as *Beauveria* spp. and thirty-five as *Metarhizium* spp. Twelve of the isolates came from hosts or substrates other than grasshoppers. Seven were isolated from the scarabaeid beetle, *Pachnoda interrupta* (Olivier), a sorghum chafer, and five from soil samples. The isolates were screened against laboratory-reared 4th instar 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 isolates, most of which were *Metarhizium* spp. Insects were dosed under the pronotum at a concentration of 1 x 10^5 conidia/ml. in 2 ul of water (200 conidia/insect) for the low dose or 2 x 10^7 conidia/ml (40,000 conidia/insect) for the high dose. At the low dose, isolates DLCO27, 40 (*Metarhizium anisopliae* var *acridum*) and 101 (*Beauveria bassiana)* gave mortalities of 65, 50, and 42%, respectively, ten days following inoculation of *L. migratoria* compared to 25% for the reference standard. Control mortality was less than ten percent after fifteen days. Despite the low actual mortality rates at the low dose, the comparatively high virulence of these new isolates warrants further investigation. At the high dose, four isolates showed asymmetrical virulence to *S. gregaria* and *L. migratoria*, respectively, at ten days post-inoculation (DLCO5, 55% vs. 15%; DLCO26, 85% vs. 65%; DLCO34, 75% vs. 30%; and DLCO91, 45% vs. 80%).

Poster / Fungi. F-22.

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 *Ornithacris 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 1.2x10^7 spores/ml aqueous spore concentrate (24,000 spores/ insect) were topically applied underneath 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 outperformed 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.

Poster / Fungi. 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 regime

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Because of severe climate conditions prevailing in areas targeted for locust control, influence of both fungus fermentation and propagule formulation was studied as a means ofr assessing blastospore quality

of *Metarhizium anisopliae* var. *acridum* (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 blastopores produced in Jackson medium without additives. Formulation assays showed that drying temperature during the dehydration phase of fresh bastospores 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 fresh 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 in contrast with the favorable 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.

Poster / Fungi. F-24.

Influence of culture conditions, nutrition, oxygen supply and pH on production of blastospores of *Metarhizium anisopliae* var. *acridum* in submerged fermentation

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Influence of nutrition conditions of the isolate IMI 330189 of *Metarhizium anisopliae* var. *acridum* (kindly provide to Virginia Tech by CABI Biosciences) was studied for assessing blastospore yields in liquid state fermentation. Based upon the early work of Jenkins and Prior (1993) the Virginia Tech Consortium (*) has been interested to develop commercially feasible method of producing *Metarhizium* propagules using liquid fermentation. Blastospores production was evaluate by microscopic counting with an hemacytometer. Culture supernatants were analyzed for residual medium sugars using HPLC. Preliminary shake-flasks experiments were employed for screening carbohydrate and nitrogen sources and for studying the influence of the C/N ratio. Maximal blastospore concentration (5.4×10^8 blastospores ml^{-1}) and faster carbohydrate consumption (total exhaustion in 72h) were observed in medium containing 2% sucrose and 2% brewer's yeast (C/N ratio of 6.5, 28°C and pH 7) The effects of oxygen supply for improving blastospore production on the submerged fermentation was studied in 5-l working volume bioreactors. Oxygen transfer was improved by both increasing aeration rates (within the range of 0.4-1 vvm) and agitation speed (130-250 rpm). The dissolved oxygen concentration (DO) was monitored by using polarigraphic DO probes. DO rates were maintained constant at 5, 50, and 100% by changing aeration (air-, N_2- and O_2- motorized valves) and agitation speed during fermentation. DO had a significant effect on the formation of blastospores. When DO was monitored at 100% of saturation, blastospore concentration was maximal, at 50% it was 1.5 times lower and at 5% or without DO regulation it was 21 times lower. Influence of pH was studied at 100% of the saturation of dissolved oxygen. Without pH regulation (initial pH7) propagule production reached to 2.3×10^8 blastospores ml^{-1}. In contrast, regulation at pH 6 or 7 caused yield enhancement up to 8.2×10^8 blastospores ml^{-1}. Under optimal conditions (C/N ratio of 1.6, regulated pH7, DO of 100%, 1-vvm flow rate, at 28°C) blastospore production reached 1.1×10^9 blastospores ml^{-1}. *Project "Development of Biopesticides for Grasshoper and Locust Control in Sub-Saharan Africa", funded by the USAID (Africa-Bureau-Funded Project Grant No AOT-G-00-97-00386-00)

Poster / Fungi. F-25.

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 hyphomycete 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 study, we selected different 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 developmental 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 *acridum* 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.

Poster / Fungi. F-26.

Epizootic potential of Trinidadian strains of *Paecilomyces 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 *Paecilomyces 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 Sheffé F-test showed no significant differences between strains in the production ratios of conidia per cadaver / blastospores ml^{-1} ($F_{2,27} = 2.2$; $P = 0.14$). 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.

Individual and combined 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 infested by *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 bean (237 ± 35 /mm^2) and geranium (218 ± 25 /mm^2). This procedure was repeated using both type of plants that were not exposed to *E. formosa*. Based on these studies, *P. fumosoroseus* is compatible with and can be used in combination with the parasitoid *E. formosa*, if exposed to *T. vaporariorum* nymphs at least four days prior to being sprayed with blastospores under laboratory conditions. Using both a parasitoid and entomopathogen seems to have potential for control of the *T. vaporariorum* on *P. vulgaris* plants in a closed system. The effect of the parasitoid and entomopathogen on the *T. vaporariorum* mortality was determined to be the result of independent actions of each agent, and not a synergistic effect for bean ($x^2 = 2.09$) and Regal geranium ($x^2 = 1.21$). For effective control of the *T. vaporariorum* infesting *P. domesticum* plants, spraying *P. fumosoroseus* blastospores alone is most efficient under laboratory conditions. Survival of parasitoids was very poor on Regal geranium plants and seems to be host plant dependent.

STU Poster / Fungi. F-28.

Zn dependence of growth and dipicolinic acid production in *Paecilomyces fumosoroseus*

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Crude broth filtrates of *Paecilomyces fumosoroseus* were toxic to whitefly nymphs. The metabolite in the highest concentration was isolated and identified as 2,6-pyridindicarboxylic acid, commonly known as dipicolinic acid (DPA). A single dosage screening showed that DPA is the main insecticidal active principle. On other hand, DPA LD_{50} on brine shrimp, *Artemia salina*, was 44.5 ± 3.1 mg/ml. Therefore, the factors affecting DPA production in submerged culture were studied.

DPA production by *P. fumosoroseus* was evaluated on different culture media. In a comparative study, logistic and Luedeking-Piret equations were used in order to estimate the following coefficients: maximal specific growth rate, $m_M = 0.0135\text{-}0.0321\ h^{-1}$, maximal biomass level, $X_M = 2.79\text{–}18.31\ g\ L^{-1}$, DPA/biomass yield $Y_{P/X} = 1.8\text{-}189.5\ mg\ g^{-1}$, and DPA rate of secondary production or breakdown, $k = 0.5\text{-}44.0\ mg\ g^{-1}h^{-1}$. DPA productivity was proportional to, $m_M, Y_{P/X}\ X_M$, corrected by a function of $n = k/Y_{P/X}\ m_M$.

In some cases, DPA was not detected while in others, DPA concentrations were up to 1 g/L. An inverse ratio between DPA production and fungal growth was found. It was shown that differences in DPA production were due to the different concentrations of Zn in the media. High Zn levels enhanced fungal growth but decreased DPA production. Estimated values of, $n \approx 0$, indicated that the DPA synthesis was directly associated to development of blastospores and/or mycelium. However, under Zn limitation, DPA production was enhanced but growth was decreased since DPA synthesis was not associated to growth process ($n > 0$). Thus, low Zn levels seem to be necessary to achieve high DPA levels in the culture broth of this organism.

Poster / Fungi. F-29.

Phylogenetic relationships of entomopathogenic fungi based on mitochondrial SSU rDNA Sequences

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Members of the Clavicipitaceae include endophytes as well as pathogens of grasses, insects and parasites other fungi. The parasitic interrelationships imply intricate mechanisms for host recognition and specificity. Therefore it is expected that through the evolutionary history these interrelationships should be conserved and recorded as genetic information. Comparative DNA sequence studies can bring new insight on the patterns of host specificity within the Clavicipitaceae group and help clarify phylogenetic relationships among them. Our purpose was to compare the sequences of the mitochondrial SSU rDNA fragments from several mitosporic Clavicipitaceae fungi (*Beauveria basssiana, B. brongniartii, Metarhizium anisopliae, M. flavoviride, Nomuraea anemonoides, N. cylindrospora, N. rileyi, N. viridulus, Paecilomyces amoenoroseus, P. fumosoroseus, P. javanicus, Tolypocladium cylindrosporum,* and *Verticillium lecanii*) to infer phylogenetic relationships among them. Isolates of these fungi were obtained from the ARSEF and from Embrapa Soja culture collections (*www.ppru.cornell.edu/mycology/insect_mycology.htm* and *www.cnpso.embrapa.br/pubonline.htm*). The DNA was extracted from mycelia using a CTAB protocol and, after cleaning and quantification, amplified with the appropriate primers. The amplified DNA was cleaned and sequenced in a ABI sequencer at the BioResource Center, Cornell University. Homologous sequences from the NCBI GenBank database, mostly from *Cordyceps* species, were included in the analysis. Two fragments, 393 and 429 bp were aligned by means of Clustal W and parsimony analysis was performed with Mega2 software; bootstrap analysis was done using 1,000 replicates. The most divergent fungus was *T. cylindrosporum* (ARSEF 963). Species of *Metarhizium* (*M. anisopliae* and *M. flavoviridae*) were closely related to *Nomuraea*, as observed by other authors when the ITS region was considered. *N. anemonoides* clustered in the same group with *B. brongniartii* and *B. bassiana*. *Paecilomyces javanicus*, *P. tenuipes*, *P. amoenoroseus* and *Cordyceps militaris* clustered in the same group. It was not possible to obtain the complete sequences of *N. cylindrospora* and *N. viridulus*, but these species appear to be more closely related to *Metarhizium* than to *Nomuraea*, thus implying that a revision of this group of genera is needed. *N. rileyi* seems to be a monophyletic group.

STU Poster / Fungi. F-30.

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/mm^2. However, infection levels were very low (0 to 5%) when *N. rileyi* isolates from GCW, SBL, FAW and BAW were tested against VBC.

Poster / Fungi. F-31.

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*), hemocytes and its phagocytosis in the hemolymph of three spices 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 spices. 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*.

Poster / Fungi. F-32.

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 *Metarhizium flavoviride* , *Aphanocladium album* , 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 *Metarhizium* are phylogenetically closely related genera.

STU Poster / Fungi. F-33.

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 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 erect phialides 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, Capnodium* 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 mycosed 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.

Poster / Fungi. F-34.

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* (1x10^8 conidia/ml) 0, 3, 5, and 7days after exposure. The mummification of aphids following spore 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 stereomicroscope and agar medium. Mummification following spore 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 maimaiga*

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Because of the mysterious appearance of the fungus in 1989, many have hypothesized that the *E. maimaiga* 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 US). We measure virulence of the isolates by injecting protoplasts into 4th instar larvae and measuring the proportion and speed of kill. Pathogenicity will be tested by immersing 4th 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.

STU 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* (Zygomycetes: 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 patterns 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.

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 *Acyrthosiphon pisum*, 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. pisum*.

Infestation of *V. faba* plants by *A. pisum* 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. pisum*. 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. pisum*-induced *V. faba* volatiles on the sporulation rate, conidium size, growth rate and, germi-nation rate of *P. neoaphidis*.

Initial results indicate no effect of *A. pisum*-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.

Poster / Fungi. F-39.

Analysis of EST sequences from the entomopathogenic alga *Helicosporidium* sp. (Chlorophyta, Trebouxiophyceae)

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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 *Prototheca*, 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.

STU Poster / Fungi. F-40.

In vitro* development of *Helicosporidium

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The protist *Helicosporidum* 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 dehisces within the midgut lumen penetrates column epithelium gaining ingress into the hemocoel. Within the nutrient rich hemolymph this pathogen undergoes multiple cycles od vegetative replication. The resulting *in vivo* cells can be harvested and cultured *in vitro*. This *in vitro* growth is characterized by the production of vegetative 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 vegetative 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 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 autosporogeny. 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*.

MICROSPORIDIA & PROTOZOA

STU Poster / Microsporidia. MP-1.

Influences of Dimilin® on a microsporidian isolate of the genus *Nosema*

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The gypsy moth (*Lymantria 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 moths 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.

Poster / Microsporidia. MP-2.

Characteristics of a microsporidian parasite, *Vairimorpha kyonggii* n. sp. isolated from *Helicoverpa armigera*

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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 spore type was found only in the muscle, while the former was found in haemocyte, nerve, fat body, silk gland, midgut, malpighian tubele, muscle, trachea and gonad. The microsporidium was transmitted to

the next generation through the egg. We conclude that the parasite is a new microsporidian species, *Vairimorpha 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, determined, 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: *Malamoeba scolyti*; Sporozoa: Eugregarina: *Gregarina typographi*, Neogregarina: *Mattesia* sp. and Microsporidia: *Chytridiopsis typographi*. The evidence of *Mattesia* sp. (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

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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 towards 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 release in the United States 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 *Chroniodiplogaster*, 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. fumosoroseus*, *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.

STU Poster / Microbial Control. MC-3.

Survey for natural enemies of the alfalfa snout beetle *Otiorhynchus ligustici* (L.) in Hungary and in New York State: *Nosema otiorhynchi*, 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 *Otiorhynchus ligustici* (L.), a serious pest introduced from Europe. The survey focused on entomopathogenic nematodes, entomopathogenic fungi, and the microsporidian *Nosema otiorhynchi* (Weiser). *N. otiorhynchi* was not detected in populations of the alfalfa snout beetle in Hungary. In New York State, a single specimen was found infected with microsporidia in Oswego County and 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. *Steinernema 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 parasitoids. 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 *Escheria ecoli* 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*. With bees from the same 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 immunosupression. Evidence for a period of immunoincompetence 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 immunoelicitors. 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 microbials 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 *Bt* for control of CPB larvae. We also investigated the effects of microbial treatments on feeding by beetle larvae and their effect on yield. Four weekly applications of *Bt*, *B. bassiana*, and a mixture of *Bt* + *B. bassiana* and alternations of the two microbials (two *Bt* treatments followed by two of *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 *Bt* alone or *B. bassiana* 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 *Bt* and *B. bassiana*. In 2000 similar levels of control were obtained with *Bt* and with the mixture of half label rates of *Bt* 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.

Poster / Microbial Control. MC-7.

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-CT97-3489) and MASTER (EU-QLK5-CT-2001-01447). http://www.rothamsted.bbsrc.ac.uk/pie/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 (-37%), while 'Hymenoptera parasitica' (+66%), spiders (+19%), and 'micro-Diptera' (+3%) were affected positively or not at all. However, the pollen beetle specific parasitoid *Phradis morionellus* was dramatically reduced in the following spring, by 94.4%, equalling 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 kIJ/sqm, *S. feltiae* in NemaBags at the rates of 450, 150 (2 dates), and 15 kIJ/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 unconducive 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 lowest rate it was possible to establish nematodes in a plot, capable of killing bait 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/pie/master/master.htm

Poster / Nematodes. N-2.

Conservation of entomopathogenic nematode populations through the manipulation of crop diversity invegetable production systems

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Diverse vegetable landscapes are plagued by a complex of pests, many of which have the potential to be managed by entomopathogenic nematodes, *Heterorhabditi*s 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/m^2. 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

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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 m^2 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

experiments include mortality rates in the two kinds of patches, host availability in the two patches, and dispersal rate functions that reflect movement either through the soil, through occasional rain events, or through phoresy or infected but still mobile insect hosts. Parameter space that leads to nematode population increase in the cultivated patches was compared with field observations.

Poster / Nematodes. N-4.

Low Cost Liquid Fermentation of Entomopathogenic Nematodes

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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 feltiae* 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.

Poster / Nematodes. N-5.

Field survey and evaluation of entomopathogenic nematodes for white grub *Phyllophaga 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 Tuxtepec (2.7 %). Drought prone areas such as Valles Centrales and Mixteca 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 feltiae* Filepjev, *Steinernema* sp., and *Heterorhabditis* sp., respectively. Lethal mean dosages ranged from 87 to 105 nematodes per white grub for *Steinernema 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 LD_{95}'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. (*Proyecto CEGEPI, IPN 988001)

STU Poster / Nematodes. N-6.

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.

Poster / Nematodes. N-7.

New strains of the entomopathogenic nematode, *Steinernema riobrave*: are they better for biological control of the citrus root weevil, *Diaprepes abbreviatus*?

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The entomopathogenic nematode *Steinernema riobrave* has proven effective against *Diaprepes abbreviatus* in certain Florida soils. However, this species is known from only a single strain, and it is possible that other strains of this species might be much more effective. Therefore, we recently returned to the lower Rio Grande Valley in Texas and Mexico where the original strain was isolated and took a series of soil samples in an effort to find new strains. These samples yielded ten new isolates of *S. riobrave* as well as a new species of entomopathogenic nematode in the genus *Heterorhabditis*. Laboratory assays comparing the new *S. riobrave* strains, the old strain, and a mixed strain (formed by pooling all ten new strains) against *D. abbreviatus* found significant differences in virulence. Under our experimental conditions (24ºC, 70% RH, Candler sand at 8% moisture in 25 dram snap-cap vials and 200 infective juveniles), and after 7 replicates of 20-30 vials per strain per replicate, the old strain produced the lowest average mortality level (57.7%) whereas the mixed strain produced the highest average mortality level (84.1%). Thus, the best and the worst strains differed by 26.4%, with the best strain killing 45.7% more than the worst strain. These data indicate that some of the new strains are considerably more virulent than the older strain and justify further testing of the new strains for characteristics important to the biological control of *D. abbreviatus* in Florida citrus.

Poster / Nematodes. N-8.

Race to death: the encapsulation response by insect hemocytes is mediated by the surface coat proteins of *Heterorhabditis bacteriophora* and *Steinernema glaseri*

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Entomopathogenic nematodes, like *Heterorhabditis bacteriophora* and *Steinernema. glaseri*, have evolved a lifecycle in which survival depends upon overcoming insect immunity for survival and repro-

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 susceptiblity. The interactions of the nematodes and the hemocytes from a series of resistant and susceptible hosts (*Manduca sexta, Galleria mellonella, Popilla japonica,* and *Acheta domesticus)* 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.

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

BACTERIA – 3

STU Contributed paper. Monday, 2:00

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

Jean-Frédéric Brunet[1,2], Vincent Vachon[1,2], Greta Arnaut[3], Jeroen Van Rie[3], Jean-Louis Schwartz[1,2,4] and Raynald Laprade[1,2]

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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 amphipathic 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 α3-α4 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 Fortier[1,2], Martin Kirouac[1,2], Vincent Vachon[1,2], Olivier Peyronnet[1,2], Jean-Louis Schwartz[1,2,3] and Raynald Laprade[1,2]

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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 Cry1Ac 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 Cry1Ac 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 Cry1Ac, 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 Cry1Ac except at high pH and very low ionic strength. At pH 10.5, increasing ionic strength decreased the activity of Cry1Ac 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.

STU Contributed paper. Monday, 2:30

Mutations in domain I interhelical loops affect the rate of pore formation by the *Bacillus thuringiensis* Cry1Aa toxin in insect midgut membrane vesicles

Geneviève Lebel[1,2], Vincent Vachon[1,2], Gabrielle Préfontaine[2,3], Luke Masson[2,3], Frédéric Girard[1,2], Marc Juteau[1,2], Aliou Bah[2,3], Benoit Rancourt[4], Charles Vincent[4], Raynald Laprade[1,2] and Jean-Louis Schwartz[1,2,3]

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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 (α4-α5 loop) were able to form pores, although W182C (α5-α6 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 (α3-α4 loop). At pH 7.5, the rate of pore formation was significantly lower for F50C (α1-α2 loop), Q151C and Y153C (α4-α5 loop), W182C (α5-α6 loop) and S252C (α7-α1a loop) than for Cry1Aa. At pH 10.5, all mutants formed pores significantly more slowly than Cry1Aa, except T122C. 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.

STU Contributed paper. Monday, 2:45

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

Frédéric Girard[1,2], Gabrielle Préfontaine[2,3], Vincent Vachon[1,2], Yanhui Su[1,2], Lucie Marceau[1,2], Aliou Bah[2,3], Luke Masson[2,3], Jean-Louis Schwartz[1,2,3] and Raynald Laprade[1,2]

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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. *medellin* (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*

J. Eleazar Barboza-Corona[1,3], Hyun-Woo Park[1], and Brian A. Federici[1,2]

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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 *cyt1A*p/STAB, yielded only a low level of Cry19A and no observable crystals. The larger crystals produced by expressing the *cry19A* operon using *cyt1A*p/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 *cyt1A*p/STAB *cry19A* operon construct was fourfold greater (LC_{95} = 1.9 mg/ml) than that of preparations produced using the wild type operon (LC_{95} = 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 mosquitocidal 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. Roy

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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 cadvers 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 ecdysteroid UDP-glucosyltransferase (*egt*) gene, influences host moulting, which in turn influences

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

John P. Burand

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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 armyworm

S.C. Cotter[1], R.S. Hails[2], J.S. Cory[2] and K. Wilson[1]

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Several insect species display altered phenology and behaviour during outbreaks, a phenomenon known as density dependent phase polyphenism. The crowded phase is typically triggered by tactile stimulation and in many locust, 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 melanism 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, 6:15

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 allantonematid 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 nematophila* and *Photorhabdus 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.

CONTRIBUTED PAPERS. Monday, 4:30-5:45.

PROTOZOA and ALGAE

Contributed paper. Monday, 4:30.

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 (trypanosomatids 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 *Mattesia oryzaephili*

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The neogregarine, *Mattesia oryzaephili*, 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 oviposite on beetle larvae that have early stage infections, and their progeny succumb to the infection. They do not oviposite 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 *Malamoeba scolyti* Purrini (Rhizopoda, Amoebidae) in different bark beetle hosts (Coleoptera, Scolytidae)

Joachim-Friedrich Kirchhoff [1], Rudolf Wegensteiner[2], Jaroslav Weiser[3] and Erwin Führer[2]

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The effects of *Malamoeba scolyti* was tested in *Dryocoetes autographus, Tomicus piniperda, Hylurgops palliatus, Hylastes ater, Polygraphus poligraphus, Pityogenes chalcographus, Pityogenes calcaratus, Ips typographus, Ips sexdentatus, Ips laricis.* Fresh *Malamoeba scolyti* cysts were yielded from infected midguts of adult *Dryocoetes 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 *Malamoeba 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.

STU Contributed paper. Monday, 5:15.

In vitro development of *Helicosporidium*

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The protist *Helicosporidum* 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 dehisces within the midgut lumen penetrates column epithelium gaining ingress into the hemocoel. Within the nutrient rich hemolymph this pathogen undergoes multiple cycles od vegetative replication. The resulting *in vivo* cells can be harvested and cultured *in vitro.* This *in vitro* growth is characterized by the production of vegetative 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 vegetative 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 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 autosporogeny. 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*.

Contributed paper. Monday, 5:30.

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, *Cyrobagus 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

generation. Injection of *Helicosporidium* spp. into the hemocoels of late instars resulted in >95% infection, suggesting that the resistance is related to the ingestion of the pathogen and therefore affiliated with midgut mediated barriers. Interestingly, the pathogen's development was not interrupted by metamorphosis nor did the infection necessarily interrupt the insects' development. When treated as early instars, 50-90% of the infected larvae formed pupa, of which 20-30% emerged as adults. However, a high proportion of the infected adults (62-86%) had malformed wings and longevity was reduced compared with healthy adults.

CONTRIBUTED PAPERS. Monday, 4:30-6:15.

VIRUSES – 2

Contributed paper. Monday, 4:30

Origins of replication in *Cydia pomonella* granulovirus

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Previous recombination experiments involving the *Sal*I-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 *Dpn*I replication assays. The *Sal*I-F fragment replicated in the CpGV-dependent DNA replication assay. A subclone of *Sal*I-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 *Pst*I-J, 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.

STU Contributed paper. Monday, 4:45

Formation of budded virus at the plasma membrane in baculovirus-infected cells involves the localisation of gp64 within lipid rafts

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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 is 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* (*Sf*9) 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 californica* nucleopolyhedrovirus (AcNPV)-infected *Sf*9 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 *Sf*9 cells were treated with methyl-ß-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.

STU Contributed paper. Monday, 5:00

RNA interference in uninfected and baculovirus-infected lepidopteran cells

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RNA interference (RNAi) is cellular mechanism capable of suppressing gene expression that has been detected in many organisms. RNAi refers to the induction of gene silencing machinery by 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 (LqqIT2) gene, or after the LqqIT2 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.

Contributed paper. Monday, 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

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Enhancins are a group of proteins first identified in granuloviruses that have the ability to enhance nucleopolyhedrovirus (NPV) potency. The first enhancin gene found in a NPV was identified in the *Lymantria dispar* multinucleocapsid NPV (LdMNPV), and was termed the *enhancin* 1 (E1) gene. A second LdMNPV *enhancin* gene (E2) was identified when the viral genome was sequenced. The E1 and E2 proteins were both shown to contribute to LdMNPV potency through bioassays of recombinant viruses lacking E1, E2, or E1 and E2. Expression and localization of the enhancin proteins were investigated through the use of polyclonal antibodies. Antibodies were generated for E1 and E2 using synthesized peptides homologous to regions of E1 and E2 that exhibit little similarity. The specificity of the antibodies was assessed using recombinant viruses that lack the *E1* gene (E1del), the *E2* gene (E2del), or the *E1* and *E2* genes (E1delE2del). Western analysis with the E1 peptide antiserum detected a protein of approximately 84 kDa in extracts from wild type and E2del infected Ld652Y cells late in infection, but not from E1del

and E1delE2del infected cells. The E2 peptide antiserum 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 enhancin 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 is there 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 a 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 they 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 iridescent* virus early and late gene

Remziye Nalçacioglu[1,2], Zihni Demirbag[2], Just M. Vlak[1] and Monique M. van Oers[1]

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Chilo iridescent 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 212 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* SPC-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'RACE 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, upstream 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 form 62 to 41 nucleotides. Changing the G to a C in a TTGTTT 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 ΔH 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 show 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^{-4} 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 in Sf9 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.

TUESDAY - 29 July

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

Genomics of entomopathogenic nematode-bacterium complexes

Symposium. Tuesday, 8:10.

EPN genomics: a suggestion for and the prospects of a genome-wide analysis of EPN dauer regulatory genes by using tools of molecular genetics elaborated in *C. elegans*

András Fodor

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

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 (synthenia) in Phylum Nematoda is rather possible although has not been studied so far. The degree of synthenia 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 synthenia 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 pathogenecity.

Symposium. Tuesday, 8:35.

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

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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*

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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 (*mrx*ACDGH) but unlike the *mrp* operon of *Proteus mirabilis*, lacked a site-specific recombinase and a *mrpB*-like gene. MrxA 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 MrxA 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 MrxA 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. Heungens, C.E. Cowles, and E.I. 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, NilD RNA (nematode intestine localization), which is required for colonization and which functions, in part, to regulate the translational efficiency of *rpoS.* NilD 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 NilD 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 b-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 NilA, NilB and/or NilC 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 NilA, NilB, or NilC 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. Felton[1], Ibrahim Ali[2] and Seth Young[2]

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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 a 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 bollworm *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,
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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, subplots 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 counted and recorded. 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

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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 s a role in virus evolution. The influence of food plant on virus infection is complex and whether these tritrophic 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

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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, heliothines treated with *Autographa 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 tetratrophic level effects on entomopathogenic nematodes

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I will discuss two systems in which trophic level effects on entomopathogenic nematodes (EPN) have been examined. The first system is the interaction between squash, the southern corn rootworm (*Diabrotica undecimpunctata howardi*) (CRW), and EPN (Barberchec1993, Barbercheck et al. 1995, Barbercheck & Wang 1996). Susceptibility to *Steinernema carpocapsae* (*Sc*) and *Heterorhabditis*

bacteriophora (*Hb*) was the highest in squash-fed CRW, followed by peanut-fed CRW, and was the lowest in corn-fed CRW. EPN-susceptibility was higher in CRW fed on bitter than nonbitter squash in 2/3 Hb strains but in 0/3 *Sc* strains. Corn-fed CRW had higher lipid contents and developed faster than CRW fed on peanut or squash. Increased EPN-susceptibility may be correlated with lower nutritional value of the host plant. On the other hand, EPN reproduction was the highest when SCRW were fed on corn and the lowest when fed on squash. Progeny number was higher in nonbitter- than bitter squash-fed CRW in 2/3 *Sc* and 1/3 *Hb* stains. Cucurbitacin D, a triterpenoid in bitter cucurbits, inhibited in vitro-growth of the symbionts of 3/3 *Sc* strains but only 1/2 *Hb* strains.

The second system involves fungal endophytes (*Epichloe*/*Neotyphodium*) that live intracellularly in the aboveground parts of turfgrass plants and produce a range of alkaloids that can enhance resistance to aboveground feeding insects. Direct effects on root-feeding white grubs are weak and variable, probably because only a fraction of the alkaloids are transferred into the turfgrass roots. However, Grewal et al. (1995) observed an enhancing effect of endophyte-infection in tall fescue and Chewings fescue grasses on speed of kill, nematode establishment, and mortality caused by *Hb* in 3rd-instar Japanese beetle, *Popillia japonica*. But many of these effects, especially increased mortality, were small and statistically not significant. Addition of ergotamine, one of the endophyte-produced alkaloids, to agar medium caused feeding deterrence, reduced weight, and increased *Hb*-susceptibility of 3rd-instar *P. japonica*. However, in greenhouse and field studies Koppenhöfer & Fuzy (2003) observed only a weak enhancing effect of tall fescue endophyte infection on *Hb*-susceptibility in oriental beetle, *Exomala orientalis*, but not in *P. japonica*.

Symposium. Tuesday, 12:10.

Interactions between nematodes, insects and other microorganisms in forest ecosystems: An assortment of symbiotic associations in detrital food webs

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In forest ecosystems, most net primary productivity flows into the detrital pathway. Detrital food webs and their trophic interactions have demonstrated the functional role of soil invertebrates in nutrient cycling and decomposition dynamics. Many forest insect species from diverse feeding guilds, foliage feeders as well as wood feeders, are involved in symbiotic associations with nematodes as well as with a variety of microorganisms such as bacteria, fungi, and yeast. The extent of these associations is broad and spans from phoresy and mutualism to parasitism/ pathogenesis. Such interactions can be inconsequential, detrimental or beneficial in the regulation of insect populations depending upon the effects of these organisms on each other. Moreover, they may affect plant health as they can suppress particular plant pathogens, or be involved in their dissemination.

The life history and ecology of many of these associations has been documented both from the plant and insect pathology perspective. However, integration of such information, particularly from a multi-species perspective that includes macroparasites and/or pathogens, has not been addressed in the context of food web dynamics. In this presentation the role of nematodes and their various symbiotic associations in detrital food webs will be illustrated. Implications of such associations in biological control will also be discussed.

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

FUNGI – 2

STU Contributed paper. Tuesday, 8:00.

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

Ayanava Majumdar[1], Mark A. Boetel[1], Stefan T. Jaronski[2], Robert J. Dregseth[1], and Allen J. Schroeder[1]

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The sugarbeet root maggot, *Tetanops myopaeformis* (Röder), is the most damaging insect pest of sugarbeet in Minnesota and North Dakota. A study was conducted in 2002 at St. Thomas, North Dakota (Pembina Co.), for managing the sugarbeet root maggot, using planting time granular and postemergence liquid applications of *Metarhizium anisopliae* strain MA-1200 with two cereal cover crops–oat, *Avena sativa* L., and rye, *Secale cereale* L. The integration of cereal covers with *M. anisopliae* 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 moderate *T. myopaeformis* 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, sugarbeet 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. myopaeformis* management. The experiment will be repeated in 2003.

Contributed paper. Tuesday, 8:15.

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

P.A. Shah, S.J. Clark[1] 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 neoaphidis*, have been performed using stratified leaf sampling techniques at two scales; a nettle bed (approx. 40 m^2) and a cereal field perimeter (approx. 900 m^2). 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 Entomphthorales populations were synchronised, appearing in May and declining to zero by July. *Pandora neoaphidis* was the most common entomphthoralean fungus in 2002 when most samples were taken. Other members of this natural enemy complex included *Entomophthora planchoniana, Neozygites microlophii, Zoophthora phallioides* and *Conidiobolus* sp. Based on quantitative data and field

observations, *M. carnosum* numbers are inflenced by aphid crowding and anthocorid predation as well as infection by Entomophthorales, while parasitism was rare at these study sites. The nettle–*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 neoaphidis*, three aphid species and different host plant resources

P.A. Shah, S.J. Clark[1] and J.K. Pell

Plant and Invertebrate Ecology Division and [†]Bioinformatics Unit, Rothamsted Research, Harpenden, Hertfordshire, AL5 2JQ, UK

Studies were carried out to compare the performance of *Pandora neoaphidis* against aphids on standard or alternate host plant species or cultivars. The three aphid species used were the pea aphid, *Acyrthosiphon 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. neoaphidis* 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 LD_{50} 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 signicantly 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.f. 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 LD_{95} dose. Infections of 9-80%, 3-32% and 25-71% were obtained with *A. pisum*, *M. dirhodum* and *M. persicae*. There were no differences in survival time between cultivars, and overall means of 3.8 - 6.5 days were computed. There was a quadratic relationship between survival time and dose, indicating non-linearity between *P. neoaphidis* performance and amount of conidia.

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

STU Contributed paper. Tuesday, 8:45.

Mycoinsecticide for stored product pest control

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The maize weevil, *Sitophilus zeamais* (Motsch.) (Coleoptera: Curculi-onidae) 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 *Metarhizium 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 talcum and conidia-based dustable 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: talcum: 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: talcum-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 formulations was possible 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 mycoinsecticieds such as the one developed in this study.

Contributed paper. Tuesday, 9:00.

Use of *Beauveria bassiana* and its environmental effects in microbial 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, 20 to 90% 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 actinomycetes 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 10^7 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.

Contributed paper. Tuesday, 9:15.

Entomopathogenic fungi and the emerald ash borer

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

(*Fraxinus* spp.) discovered May 2002 in southeast Michigan, then in Ontario, Canada, and most recently in northwestern Ohio. Larval feeding in the cambium of ash trees leads to rapid host decline and death in ca. one to three years; millions of ash trees are now dead and dying. We began a survey of EAB natural enemies in Michigan during July 2002 by removing EAB from infested ash trees; live larvae were rearing to adult; dead larvae were cultured for fungi or necropsied for cause of death; parasitoids and predators were reared to adult for later identification. Fungi were the most prevalent natural enemy of the larval stage including isolates of *Beauveria bassiana*, *Paecilomyces* spp., *Metarhizium anisopliae,* and *Verticillium lecanii*, in decreasing order of abundance. We conducted laboratory bioassays to compare the infectivity and virulence of several fungal isolates against EAB. Larval and adults were susceptible to infection by all the isolates of *Beauveria bassiana* and *Metarhizium anisopliae* tested, with significant inter- and intra-species differences; adults were more susceptible to infection than larvae. The most virulent fungal isolate was *B. bassiana* GHA, the active ingredient of the registered bioinsecticides Mycotrol ES® and Mycotrol O®. EAB adults died within ca. 6-10 days at the LC_{50} of these products tested on ash leaves and bark. We will discuss potential strategies for use of fungal insecticides in the management or eradication of EAB.

Contributed paper. Tuesday, 9:30.

Impact of different components of entomopathogenic fungi on the hemlock woolly adelgid, *Adelges tsugae* Annand (Homoptera: Adelgidae)

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Conidia, blastospores and cultural liquor after submerge cultivation of two species of fungi, *Beauveria bassiana* (*Bb*), strain CA 603, and *Verticillium lecanii* (*Vl*), strains HWA 304 and SPTR 151 were examined for their potential as biological control agents for an important forest pest, the Hemlock Woolly Adelgid (HWA). All fungal components were tested separately and in different combinations. The test concentration for all treatments was fixed at 10^7 propagules per ml, and the cultural liquor was diluted 1:1 prior to application. All experiments were conducted July 2002 in the forest with the aid of a specialized 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.l.* strains HWA 304, isolated from HWA and SPTR 151 isolated from *Eurygaster integriceps* (*Hemiptera: Scuterelleridae*) 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 liquor 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.l.* strain HWA 304 and 100% for *V.l.* strain SPTR 151. The high effectiveness of blastospores in cultural liquor 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 liquor 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.

STU Contributed paper. Tuesday, 9:45.

Effect on bloodmeal size and egg production of the malaria mosquito *Anopheles gambiae s.s.*, when infected with *Metarhizium anisopliae*

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In previous studies we showed that the entomopathogenic fungus *Metarhizium anisopliae* is pathogenic to the malaria vector *Anopheles gambiae s.s.*. In the work presented here we studied two effects of the fungus on female mosquitoes, when contaminated with a medium lethal conidial dose: bloodmeal size and egg production. Mosquitoes were passively contaminated with oil-formulated conidia and were offered human blood either 1, 2, or 3 days after the fungal contamination. Results show that fungus-infected female *An. gambiae* mosquitoes take up less blood and produce fewer eggs. Whether these results will have any implications regarding malaria transmission risk if this fungus is used in mosquito management strategies will be discussed.

Contributed paper. Tuesday, 10:00.

Why fat bees get chalkbrood

R. R. James[1] and James Buckner[2]

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Ascosphaera aggregata is an Ascomycete that causes chalkbrood disease in larvae of the alfalfa leafcutting bee, *Megachile rotundata* (Hymenoptera: Megachilidae). The *Ascosphaera* are different than most entomopathogenic fungi in that infection initiates in the host midgut. Germination requirements for this obligate pathogen are complex and not fully understood. CO2 is required for germination, but it is not sufficient when to induce germination on general purpose fungal nutrient agars. The addition of canola oil to a specialized agar has been reported to induce spore production in vitro, and so we tested whether lipids might help induce spore germination as well. We found *M. rotundata* larvae to have very high lipid contents (20.6%) relative to other insects. We tested plant (canola) lipid and lipids extracted from bee larvae for their effects on germination. After 24 h, germination in the presence of lipids significantly increased. More germination, with less variance, occurred when bee lipids were used. *A. aggregata* spores tended to attach to the lipid droplets in shaken cultures, and germination was significantly greater among spores which had attached. Thus, lipids in the larval gut, or in tissues of the gut, help stimulate germination during initiation of infection.

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

VIRUSES – 3

Contributed paper. Tuesday, 8:00.

The infection and pathogenesis of the *Amsacta moorei* entomopoxvirus in *Lymantria dispar* larvae

Basil Arif[1], Qianjun Li[2], Lillian Pavlik[1], Richard Moyer[2]

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The infection and pathogenesis of *Amsacta moorei* entomopoxvirus (AmEPV) in gypsy month (*Lymantria dispar*) larvae were investigated following ingestion of virus-infected cells or injection of 300 viral plaque-forming units directly into the haemolymph. These studies were facilitated by use of the AmEPV(Sph+20)::GFP, a recombinant virus that expresses GFP under the control of late *spheroidin* promoter. Shortly after inoculation, virus replication in the haemocytes was observed, followed by subsequent involvement of other tissues, including the silk gland, fat body, hindgut and midgut. The malpighian tubules were the last tissues to be infected with virus.

Detailed examination of the trachea 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 ATI promoter was also tested for its growth and pathogenesis in gypsy month 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 infectivity factor, PIF-1 (Ac119 homologues). These products are associated with the envelopes 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, ptpt-2, egt, pkip-1, and arif-1). These genes appeared thus not essential for virus replication in cell culture, nor did they affected virus replication in insects as evidenced by *in vivo* intrahemocoelic 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 Pijlman *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 was generated. Analyses of these mutants indicated that a deletion of Se35 results in the loss of oral infectivity of polyhedral occlusion bodies. Reinsertion 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* NPV, 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.

STU Contributed paper. Tuesday, 8:30.

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

Serafin Gutiérrez[1], Ohiane Simón[2],
Primitivo Caballero[2] and Miguel López-Ferber[1]

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PIF is a baculovirus protein essential for oral infectivity of occlusion derived virions (ODV) in lepidopteran larva. 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 SpliNPV *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 (SpfrNPV). Plaque assay analysis of a wild SpfrNPV 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.

STU Contributed paper. Tuesday, 8:45.

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

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The genome of *Jc*DNV 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 *Jc*DNV in insect cells was previously reported (Croizier *et al.,* 2000). However, the role of each polypetide 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 in-frame ATG's at position 555 (pJ□VP1), 1386 (pJ□VP2), 1521 (pJ□VP3), 1668, and 1674 (pJ□VP4) Plamid 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 VP3 in virus assembly and infectivity and confirm the essential role played by VP4 in virus morphogenesis and by VP1 in virus infectivity. Ref. : Croizier L., Jousset F.X., Veyrunes J.C., Lopez Ferber M., Bergoin M., Croiziez G. (2000). *J. Gen.Virol*, **81**, 1605-1613.

STU Contributed paper. Tuesday, 9:00.

Analysis of a zinc-finger protein from *Choristoneura fumiferana* nucleopolyhedrovirus

Jondavid de Jong [1], Basil Arif [2] and Peter Krell [1]

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The *Choristoneura fumiferana* multicapsid nucleopolyhedrovirus (*Cf*MNPV) is an ideal candidate as a bioinsecticide to control the eastern spruce budworm (*C. fumiferana*) due to its narrow host-range. We have identified a *Cf*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 *Cf*-203 cells. Viral growth curves and temporal expression analyses have indicated that the null-mutant behaved similarly to wild-type *Cf*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

Yi Huang[1], Hanzhong Wang[1], Xinwen Chen[1], Li Yuan[1], Just M Vlak[2], Zhihong Hu[1]

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During the construction of Bac-to-Bac system of *Helicoverpa armigera* single nucelocapsid nucleopolyherovirus (HaSNPV), many bacmids with large deletions in the genome were obtained. Here we report the study of one of the bacmids, named HaBacHZ11, 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 HaBacHZ11 and generated recombinant HaBacHZ11PHeGFP. The DNA of the recombinant was transfected into HzAM1 cells, occlusion bodies and green fluorescent were found in the HzAM1 cells in 5-7 days after transfection. One-step growth curve of the recombinant virus indicated that HaBacHZ11 was infectious to HzAM1 cells. The electron micrographs analysis revealed large amount of virions were produced in the nucleuses of the infected cells, but they were not properly occluded into polyhedra. Bioassay was conducted with polyhedra of HaBacHZ11PHeGFP in comparison with that of HaBacHZ8PHeGFP and wt-HaSNPV by oral infecting *H. armigera* larvae. Although HaBacHZ11PHeGFP had a higher LD_{50} and a lower ST_{50} 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 cell 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-liberated occlusion body-derived virus (ODV). Cell lines from *Spodoptera frugiperda* (IPLB-Sf21AE), *Anticarsia gemmatalis* (UFL-Ag286), *Heliothis virescens* (IPLB-HvE1a, IPLB-HvE6a, IPLB-HvE6s and IPLB-HvT1), *Lymantria dispar* (IPLB-LdEIta and IPLB-LdEp), *Plutella xylostella* (IPLB-PxE2), and *Trichoplusia ni* (TN-368, IAL-TND1, and IPLB-TN-R^2) in 96-well tissue culture plates were each infected with dilutions of ODV from three nucleopolyhedroviruses (NPVs), including *Autographa californica* NPV (AcMNPV), *Anagrapha 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 resulting 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.

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

Challenges to the use of fungi for control of Acari

Symposium. Tuesday, 10:30.

Laboratory and glasshouse evaluation of entomopathogenic fungi against the twospotted spider mite, *Tetranychus urticae*

David Chandler[1], Gillian Davidson[1] and Rob Jacobson[2]

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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 phytoseiid mites. However, this is often not effective on its own, and supplementary acaricide sprays are used routinely. On crops such as tomato, differences in 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.

Symposium. Tuesday, 10:54.

Challenges in using *Neozygites tanajoae* as a classical biological control agent for the cassava green mite in Africa

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In the past 14 years, Brazilian isolates of the fungus *Neozygites tanajoae* (Zygomycetes: Entomophthorales) have been investigated as a control agent for the cassava green mite (CGM), *Mononychellus tanajoa*, 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. tanajoae* occur in parts of Africa makes the implementation of a control program challenging. Moreover, *N. tanajoae* 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* culture is still 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. tanajoae* in Africa because this pathogen presents desired attributes of a classical biological control candidate. *N. tanajoae* 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. tanajoae* 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.

Symposium. Tuesday, 11:18.

Novel strategies for control of chicken mites (*Dermanyssus gallinae*) using autodissemination

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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-infect 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 10^8 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.

Symposium. Tuesday, 11:42.

Fungi for control of ticks

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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 unfed 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.

Symposium. Tuesday, 12:06.

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

Gillian Davidson[1], Caroline Birchall[2], Judith Pell[2], Brenda Ball[2], Keith Sunderland[1] and David Chandler[1]

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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 10^8 ml^{-1}) at 25ºC / 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 30ºC / 40% RH (1 x 10^8 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 10^6 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 *V. 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.

Tuesday,10:30-12:30.

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 studing the genomic diversity of the egyptian densovirus isolate from *Mythimna loreyi* (*Ml*DNV), 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 25nm icosahedral nonenveloped DNA virus sharing the main biological and biophysical properties of densovirus that we named *Ha*DNV. After characterization and partial cloning of the genome, the presence of antigenic cross-reactivity and some sequence homology indicates that *Ha*DNV and *Ml*DNV are not phylogenetically distant. The 6 kb genome of *Ha*DNV was found to have high homology with members of the Densovirus genus as *Galleria mellonella* DNV, *Junonia coenia* DNVand *Ml*DNV.

STU 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 parvovirus 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 parvoviruses, *Galleria mellonella* and *Mythimna loreyi* densoviruses, *Gm*DNV and *Ml*DNV, 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, *Gm*DNV is restricted to its host *Galleria mellonella* whereas *Ml*DNV is polyspecific infecting several insect species within order Lepidoptera. Using two infectious clones, pGm4 derived from *Gm*DNV (Tijssen et al., in press) and pMl28 derived from *Ml*DNV (Fédière, 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 *Ml*DNV was polyspecific, infecting the three cell types, whereas *Gm*DNV 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 chimerae 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 *Gm*DNV clones to SF-9 cells. Whereas, exchanging the entire *Gm*DNV-VP sequence by its homologue of *Ml*DNV, 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 aa 1 and aa 193. That region of *Gm*DNV-VP, which contains 27 different amino acids from that of *Ml*DNV, 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 GVs than to those from NPVs. 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 lef-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 AcMNPV 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 (SlNPV), encodes a 49 kDa apoptosis suppresor protein which displays 48.8% identity to P35 of AcMNPV. Computer-assisted modeling of P49 based on the structure of P35 predicted seven α-helical motifs, three of them unique to P49. The structure includes a reactive site loop (RSL) protruding from a β-barrel domain that begins at the α1 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 α-helical regions α1, α2 and α4 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.

Hz-2V genome analysis

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Hz-2V is a viral pathogen, which causes the sterility in *Helicoverpa zea* moths. Hz-2V is a non-occludded, 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 Hz-1 virus in DNA sequence homology and genomic organization. The G+C content of Hz-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 (pdv-e56, p74, and p91) while the Hz-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 Hz-2V genome including carboxylesterase, ribonucleotidereductase, serine, deoxyribonucleosidereductase, dihydrofolatereductase, and zinc metalloprotease from *Drosophila*, *Aedes* and *Heliothis*. Hz-2V also contains several additional baculovirus gene homologues (AcORF-22, AcORF-98, AcORF-119, PxORF-109, and PxORF-29), however, the relationship between Hz-2V and baculoviruses based on gene content and organization, is still unclear.

Poster / Viruses. V-6.

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

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Replication of Hz-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 oviducts and proliferation of the cells that comprise these tissues. To determine when during development, this malformation occurs we have examined tissues destine 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 Hz-2V

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Hz-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.

Poster / Viruses. V-8.

The open reading frame 132 of *Helicocoverpa armigera* single nucleopolyhedrovirus is not essential for viral replication *in vitro*

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The open reading frame 132 (Ha132) of *Helicoverpa armigera* single nucleocapsid nucleopolyherovirus (HaSNPV), a homologue of AcMNPV ORF22, is conserved among baculoviruses whose genomes have been sequenced so far. To elucidate its function we investigated its transcription, expression. Sequence analysis indicated that *Ha132* is 1152 bp long and encodes a putative protein of 384 amino acids with a predicted molecular size of 44.5 kDa. Alignment of HA132 and its baculovirus homologues revealed that HA132 was highly conserved among baculoviruses, there were 88 conserved amino acids and 14 absolutely conserved Cysteine residues between HA132 and its homologues. Computer-assisted analysis revealed there was a signal peptide near the N-terminal of this protein. Northern blot results suggested *Ha132* is a late gene and produced multiple transcripts in different sizes. To elucidate its function, *Ha132* was expressed as a GST-fusion protein in *E. coli*. The expressed protein was purified and used to generate antibodies in rabbits. Western blot analysis of extracts of HaSNPV–infected HzAM1 cells revealed a specific protein of 43 kDa from 48 h to 96 h p.i. To investigate whether HA132 is a structural component of HaSNPV, Western blot analysis of proteins in budded viruses (BVs) and occlusion derived virions (ODVs) was conducted. The protein was not detected either in ODV or in BV, suggesting that HA132 is not a major structural component of HaSNPV. To elucidate the function of this gene, a *Ha132* deletion mutant (HaSNPVΔ132) was generated using the l-phage Red recombination system in *E. coli*. Electron microscope pictures revealed the deletion virus could replicate in HzAM1 cells, which indicates *Ha132* is not essential for the replication of HaSNPV in cell culture.

STU Poster / Viruses. V-9.

Differential activity of *Helicoverpa armigera* nucleopolyhedrovirus on cotton, chickpea and tomato

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As insecticide resistance increases microbial pesticides are becoming an ever more important alternative in the control of agriculturally important insect pest populations. However, the success of microbial insecticides to control insect pests has been limited in part because of the wide variations in persistence or efficacy among different crop plants. Understanding the factors that affect persistence will help identify approaches that enhance the efficacy of biopesticides**.** The purpose of the present study was to assess the effect of 3 host plants (chickpea, *Cicer arietinum* L., tomato, *Lycopersicon esculentum* and cotton, *Gossypium hirsutum*) on the efficacy of the *Helicoverpa armigera* nucleopolyhedrovirus (*Hear*NPV). The role of plant surface factors or inter- and intracellular leaf components in changes to the efficacy of the *Hear*NPV was investigated by spraying virus on to the leaf surfaces of the three crops for 1 and 24 hours and then washing off and testing in artificial diets against *Helicoverpa armigera* larvae. *Hear*NPV was completely inactivated when sprayed on to the leaf surface of chickpea indicating that leaf surface factors were responsible for the effect. Conversely surface factors did not affect

the efficacy of *Hear*NPV 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*NPV was unaffected by exposure to the leaves of tomato. Studies are currently underway to determine the component(s) responsible for *Hear*NPV inactivation using selective bioassay and analytical chemistry. The qualification of the *Hear*NPV 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 haemocele. 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 LC_{50} 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-insecticides. 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 important effects 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 polyhedron 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 *Helicoperva armigera* SNPV. Signals of hybridization were produced for *Hind*III-R/*Hind*III-S (comigrated in the gel), *Eco*RI-B, *Bst*EI-B and *Pst*I-A fragments of AgMNPV. The complete nucleotide sequence was performed by dye terminator chemistry method after subcloning fragments of a viral *Hind*III library. The *25K FP* gene opening reading frame of AgMNPV is 627 bp, encoding for 208 amino acids. It was found that 94 bp of the gene is located in the *Hind*III-S fragment (N-terminal) and 530 bp in the *Hind*III-R fragment (C-terminal). The following identities were obtained by amino acid sequence alignment of 25K FP protein from other nucleopolyhedroviruses: 87% with *Epiphyas postvittana* MNPV, 83% with Orgyia *pseudotsugata* MNPV, 75% with *Autographa californica* MNPV, *Rachiplusia ou* MNPV and *Bombyx mori* MNPV and 60% with *Helicoperva 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 Tn5B1-4) 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 H5Cl-B and H5Cl-F, exhibited a distinct morphology and a similar growth rate in serum-containing TNMFH medium. Clone H5Cl-B was remarkably resistant to nutrient stress in phosphate buffered saline while both clones were highly resistant to Actinomycin D. Both H5Cl-B and H5Cl-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 H5Cl-B and H5Cl-F clones could provide significant application in large-scale production of recombinant proteins and wild-type viruses.

Poster / Viruses. V-13.

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 (eEFs) 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 eEF2, 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 eEF2 were shown to contain a 5' terminal oligopyrimidine (TOP) tract. According to our data, using eEF2 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 RNAs, via a yet unknown mechanism, appears to be the main regulating factor in baculovirus-induced host shut off.

Poster / Viruses. V-14.

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

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A novel baculovirus (*Hh*MNPV) has been isolated from the neotropical butterfly *Heliconius himera*. Electron microscopy of *Hh*MNPV-infected tissues indicated that nucleocapsids of this virus are multiply enveloped and are embedded in relatively small occlusion bodies. Although *H. himera* larvae are highly susceptible to infection by *Hh*MNPV, they do not display the typical terminal wilting symptoms associated with many baculovirus infections. *Hh*MNPV 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 *Hh*MNPV.

Poster / Viruses. V-15.

Purification and charcaterazation of two viral particles from diseased postlarvae of *Macrobrachium rosenbergii*

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A disease of *Macrobrachium rosenbergii*, the giant fresh water 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 successfully performed by sucrose and CsCl gradient density centrifugation. The larger particle had a mean buyant density of 1.32 g/cm^3 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 buyant density of 1.33 g/cm^3 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 clakii* is one of alternative hosts of WSSV. In this study, viral propagation ability in circular haemocytes of crayfish *Procambarus clakii* have been investigated. Circular haemocytes of WSSV infected animals and WSSV inoculated haemocytes were collected, fixed and sectioned, then observed by routing 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 demostrated 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 clakii*. 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 gravely 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 nouvel 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.

Poster / Viruses. V-18.

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*

al., 1989) and *pif* (Kikhno *et al*., 2002). P74 was suggested to block binding of ODVs to cellular membranes (Faulkner *et al*., 1997), while the action of PIF remains not clear.

Analysis of midgut virus-specific transcription by RT-PCR on midgut cells after infection with *pif* defective baculoviruses did not allow to detect any specific viral transcript, suggesting PIF implication in the early steps of infection, binding and fusion.

Absence of PIF protein in the envelope of ODVs did not prevent binding. ODVs were observed by electron microscopy in contact with the microvilli. In addition, no nucleocapsids were detected inside the microvilli. Time course on the fusion using labelled ODVs by confocal microscopy confirmed the previous findings: no increase in the R18 fluorescence was detected on PIF deleted viruses, while the fluorescence increases when wild type ODVs were used. All those data suggest that PIF is involved in the fusion process.

Poster / Viruses. V-19.

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-exposure. 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, 8, 12, and 24 hr periods. Percent infections 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 (822 amino acids, MW of 93 kDa) has been identified as the major occlusion body protein of CuniNPV with no homology to any other known baculovirus gene. 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.

Poster / Viruses. V-20.

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

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A nucleopolyhedrovirus of *Anticarsia gemmatalis* (AgMNPV) has been widely used as a microbial insecticide in soybean in Brazil. Over 1.6 million hectares have been treated with the AgMNPV in the season 2002/2003. The possibility of selecting field populations of *A. gemmatalis* resistant to the AgMNPV has been a concern. Although this phenomenon has not been detected in the field yet, a laboratory population of the insect submitted to selection pressure was selected for high resistance to this virus. The insect midgut is considered one of the most effective barriers against viral invasion into the hemocele. Our work aimed to verify if there are differences in the midgut structure, comparing the ultrastructure of the epithelial cell surface of uninfected susceptible (SL) and resistant (RL) *A. gemmatalis* 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 region of 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 columnar cell surface along the midgut of SL and RL of *A gemmatalis*, which may be related to the insect resistance to AgMNPV infection. This work has been supported by FAPESP and PRONEX (MCT/Finep/CNPq).

Poster / Viruses. V-21.

Is the *Nucleopolyhedrovirus* of *Anticarsia gemmatalis* (AgMNPV) ineffective to infect AgMNPV resistant host larva midgut cells?

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Anticarsia gemmatalis, is a key pests of soybean in Brazil. It has been controlled by a nucleopolyhedrovirus (AgMNPV), which is widely used as a microbial insecticide in the country. The constant and increasing use of this biological insecticide in some regions have caused concerns about the possibility of selection of viral 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 AgMNPV. 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 hemocele. Our work aimed to verify whether or not the AgMNPV 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 Soja, Londrina-PR, Brazil. The AgMNPV used as inoculum was incorporated into the insect diet at 60,000 occlusion bodies/ml of diet. The midguts were collected up to 120h post infection, processed and analyzed under transmission electron microscopy. The columnar cells were the most affected ones among the different midgut epithelial cells. In the SL, these cells showed many morphological signs of cellular damage from 24 hours after infection, mainly at the proximal midgut region. However, in RL these cells exhibited minor morphological damage up to 120 hours. Polyhedra were visualized in the midgut of both SL and RL from 96 hours of infection, but the amount of viral structures were always higher in SL, affecting the midgut epithelial and tracheal cells, as well as attached hemocytes (mainly plasmatocytes and granulocytes). Our results showed that the AgMNPV, in fact, invaded RL cells. However, the virus did not affect these insects as they did with the susceptible ones, allowing development and survival of resistant larvae. The mechanism interfering with the virus infection progress in

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. ipsilon* NPV

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The common cutworm *(Agrotis segetu*m) and the black cutworm *(A. ipsilo*n) are serious soil pests of many vegetable and field crops all over the world. We have demonstrated the cross-infectivity of two baculoviruses, *A. segetum* nucleo-polyhedrovirus (AgseNPV) and *A. ipsilon* nucleopolyhedrovirus (AgipNPV) 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 three 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. ipsilo*n.

Poster / Viruses. V-23.

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

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Viral chitinase (*chi*A) 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 liquefaction of CpGV infected larvae cadavers was observed.

Poster / Viruses. V-24.

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

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The baculovirus AcMLF9.ScathL 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.ScathL, 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.ScathL or AcMNPV C6. Control regimes consisted of *Sitotroga cerealella* (Olivier) eggs for *C. carnea*, and *Ostrinia nubilalis* (Hübner) eggs and aphids for *C. maculata*. Survival of *C. carnea* fed *Sitotroga* eggs and AcMLF9.ScathL-infected *H. virescens* was significantly higher than for *C. carnea* fed *H. virescens* that were uninfected or infected with AcMNPV C6. There were no significant differences in development rates between *C. carnea* fed *H. virescens* infected with AcMNPV C6 or AcMLF9.ScathL. Baculoviruses ingested by *C. carnea* larvae remained viable within the digestive tract until adult emergence but had no detrimental effect on egg production. There was no evidence of adverse effects of AcMLF9.ScathL on *C. maculata* although this species exhibited low survival on diets composed exclusively of *H. virescens*. In choice tests, neither predator exhibited a preference between uninfected *H. virescens* and *H. virescens* infected with AcMNPV C6 or AcMLF9.ScathL. The data suggest that use of AcMLF9.ScathL in pest management would pose no greater risk to insect predators in the environment than use of the wildtype virus AcMNPV 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 *Anomala cuprea* entomopoxvirus (AcEPV), 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.

Poster / Viruses. V-26.

Expression of a *Toxoneuron 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 vSynVI-gal and the transfer vector pSynXIV. 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.

Poster / Viruses. V-27.

Picorna-like viruses in honey bees: transmission routes and role of *Varroa* mites in infection

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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) and sacbrood virus (SBV). Based on genomic sequence information 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.

STU Poster / Viruses. V-28.

DNA polymerase sequence analysis and host range of *Ascovirus* 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. zea,* Sf82-126 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. zea*) was the same as a *Spodoptera frugiperda* isolate (Sf -82-126) from Georgia. DNA sequence analysis of the DNA polymerase gene of INDO1, INDO2 and SC4 showed that INDO1 and SC4 have 99% similarity with SfAV1, and INDO2 has 91% similarity with HvAV3. The translation of the ORF within these INDO 1 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 INDO1 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.

STU Poster / Viruses. V-29.

Determination of PhopGV activity by a precise surface contamination method

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Phthorimaea operculella granulovirus (PhopGV) is the main component of viral bio-pesticides used to control the various potato tuber moth (PTM) species. *P. operculella* (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. operculella* larvae obtained with 5 different concentrations (0.6 to 600 granules/mm^2) ranged between 10 to 100%. The LC_{50} was determined to be 9 granules/mm^2. With this technique, the use of 10^6 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 10^9 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. operculella* laboratory colonies.

BACTERIA

STU Poster / Bacteria. B-1.

Endospore degradation in an asporogenic, crystalliferous mutant of *Bacillus thuringiensis*

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An asporogenic, crystalliferous 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 afected 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 new high efficiency 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* □-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–*Helicovera 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 fiftieth 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% b-exotoxin can provide control of *H. armigera* on cotton in climatic condition of the Zeraphshan valley.

Poster / Bacteria. B-4.

The research and developement 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 □-exotoxin. PCR and RFLP analysis exhibit that the strain contains nine types of ICPs gene : *Cry1Aa, Cry1Ac, Cry1Ca, Cry1D, Cry1I, Cry2A, Cry2B, Cry9E*, 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/μl *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.

STU Poster / Bacteria. B-5.

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), *Heliothis virescens*, and *Helicoverpa 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.

Poster / Bacteria. B-6.

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, cry1B* 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 *cry11* 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-7.

***Aedes aegypti* larval control with *Bacillus thuringiensis* serovar. *israelensis*: long lasting effects of an experimental tablet formulation**

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The residual mosquitocidal activity of the experimental tablet formulation XL-47 based on *Bacillus thuringiensis* serovar. *israelensis* (Bti) was evaluated against *Aedes aegypti* larvae under indoor sun light exposure field simulated conditions. Plastic containers were filled with 50 l of water and set close to a window, were they received direct sun light during half a day. Fifty, first instar, and 50 third instar *Ae. aegypti* larvae were placed in each container. Treatments were defined as 0, 3, 5 and 8 tablets per container, and each treatment was set by triplicate. Twice a week, 50 first instar larvae were added to each container and the activity of the tablet formulation was estimated by daily collecting the pupae produced. On weeks 17 and 22, by duplicate, 50 ml water samples were collected from the top, middle and bottom parts of each container. By duplicate, 5 ml of the water samples were used to set bioassays with 5 first instar *Ae. aegypti* larvae, and mortality was scored after 24 h. In order to estimate the Bti population in the water column, 100 µl of water samples were plated on nutrient agar by triplicate. Plates were incubated for 24 h at 30°C before assessing colony forming units (CFU). According to the CFU and mosquito larvae bioassays results, the spores and the active ingredient of the tablet formulation remained at the bottom of the containers with mosquito larvae reaching the formulation by diving and shredding the tablet´s material. In all Bti treatments, percent reduction of pupae formation was 100% during 12 weeks, and over 84% during 16 weeks.

STU Poster / Bacteria. B-8.

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, cry4Ba, cry10Aa, cry11Aa* and *cyt1Aa*, all reside on the 128 kbp plasmid pBtoxis [1]. Three (excluding *cry4Ba* 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 *cyt1Aa*, *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 P_{psbA} and P_{A1} [3], it displayed highest toxicity ever achieved in transgenic cyanobacteria against 4th-instar larvae of *A. aegypti*. Cyt1Aa 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.

This organism, which serves as a food source to mosquito larvae and could multiply in their breeding sites, may solve the environment-imposed limitations of *Bti* as a mosquito biological control agent. [1] Berry, C., O'Neil, S., Ben-Dov, E., Jones, A. F., Murphy, L., Quail, M. A., Harris, D., Zaritsky, A. & Parkhill, J. (2002). Appl. Environ. Microbiol. **68**: 5082-5095. [2] Khasdan, V., Ben-Dov, E., Manasherob, R., Boussiba, S. & Zaritsky, A. (2001). Environ. Microbiol. **3**: 798-806. [3] Xiaoqiang, W., Vennison, S.J., Huirong, L., Ben-Dov, B., Zaritsky, A. & Boussiba, S. (1997). Appl. Environ. Microbiol. **63**: 4971-4975.

STU Poster / Bacteria. B-9.

Toxicity against larvae of *Aedes Aegypti* and synergism with Cry toxins by 3 different Cyt proteins from *B. thuringiensis*

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The larvicidal activity of *Bacillus thuringiensis* subsp. *israelensis* (*Bti*) against mosquito and blackfly larvae is included in the following five major polypeptides of the parasporal crystalline body (d-endotoxin) produced during sporulation: Cry4A, Cry4B, Cry10A, Cry11A and Cyt1Aa (of 134, 128, 78, 72 and 27 kDa, respectively). Cyt1Aa is least toxic but is most synergistic to any of the others and their combinations. Cyt1Aa 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 *Bti* retain their original sensitivity levels in the presence of moderate concentrations of Cyt1Aa, thus playing a critical role in suppressing resistance to Cry toxins.

Here, we compared the levels of toxicity and synergism of the three known Cyt toxins from *Bti*, Cyt1Aa, Cyt2Ba and Cyt1Ca. Each of the respective *cyt* was cloned alone in expression vector and over-expressed in *Escherichia coli*. They yield diverse toxicity levels against *Aedes Aegypti* larvae and synergism by various combinations of *Bti* Cry's expressed in transgenic *E. coli*.

Poster / Bacteria. B-10.

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.

Poster / Bacteria. B-11.

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 comparation 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.

Poster / Bacteria. B-12.

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 was 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 to mummy 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.

Poster / Bacteria. B-13.

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 tracer 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 3-5cm below the soilsurface. 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 upwind direction is the maximum dispersal area. Concentration of WG-001 on cotton leaves decreased continuously and no WG-001 colony can 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 bacterial populations fluctuated at the range of 3.9 x 10^5 cfu/g soil and 4.2x10^5 cfu/g soil and the range of the total numbers of indigenous fungi populations is 1.2 x 10^5 cfu/g soil to 1.7 x 10^5 cfu/g soil. As a result, there is a restriction of the diffusive distance by airstream and survivality 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 Hi-Tech 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 lepidopteran, *Manduca sexta*. This insect has been invaluable as a model for investigating Cry1 toxin mode-of-action. Protease-activated Cry1Ac binds 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 glycosylphosphatidyl inositol (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), *Artocarpus integrifolia* (jacalin), *Ricinus communis* (RCA), *Ulex europeaus* 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.

APN was treated with PNGase F resulting in a decrease in molecular mass. After deglycosylation, APN lost most of its Cry1Ac binding capacity. APN was also treated with a GalNAcase, which reduced toxin recognition of the protein. These results suggest that the binding determinant is an oligosaccharide containing terminal GalNAc, or a very close related sugar involved in the recognition process.

Poster / Bacteria. B-15.

Role of HevCaLP knockout in alteration of Cry1A toxin binding in Bt-resistant *Heliothis virescens* strains

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A previous report demonstrated that disruption of a cadherin-superfamily gene in the YHD2 strain of *Heliothis virescens* is linked to high levels of resistance to Cry1Ac. This gene disruption results in the absence of a cadherin-like protein (HevCaLP) in the midgut epithelium of resistant larvae. Brush border membrane vesicles (BBMV) prepared from YHD2 midguts had altered Cry1Aa toxin binding when compared to susceptible vesicles, suggesting that HevCaLP could function as Cry1Aa toxin receptor. Previous competitive binding studies indicate that in *H. virescens* BBMV, all the Cry1Aa binding sites also bind Cry1Ab, Cry1Ac, and Cry1Fa. Therefore, lack of HevCaLP in BBMV would explain cross-resistance to all these toxins as observed in YHD2 larvae. In a previous report we hypothesized the existence of a similar mechanism of resistance in larvae from a different Cry1Ac-resistant *H. virescens* strain called KCBhyb. Despite the evidence for the importance of HevCaLP for toxin binding and resistance, the potential role of HevCaLP as toxin binding site has not been addressed. Using a diagnostic PCR approach, we obtained the HevCaLP genotype of individual midguts from susceptible (YDK) and resistant (YHD2, KCBhyb, CXC) *H. virescens* strains. All tested midguts from the YHD2 strain were homozygous resistant (rr) for the cadherin gene disruption, as expected. Most tested midguts from the YDK and CXC strains were homozygous susceptible (ss) but some were heterozygous (rs), which was not expected. KCBhyb had all three genotypes in approximately equal proportions. Using Cry1 toxin binding assays with BBMV prepared from the genotyped midguts we were able to study the correlation between cadherin gene disruption and alteration of Cry1 toxin binding in resistant *H. virescens* strains. Our results provide evidence for the role of HevCaLP as the primary Cry1A Poster (bacteria)

Poster / Bacteria. B-16.

Identification of a toxin-binding protein involved in resistance to Cry1Ac in *Heliothis virescens*

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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 BBMV 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 BBMV, absence of this sugar in toxin binding glycoproteins from BBMV of YHD2 insects may account for decreased Cry1Ac binding to these vesicles. Our main goal was to identify the proteins with altered glycosylation in BBMV from YHD2 and study their role in Cry1Ac mode of action. Competition of SBA binding to the 63- and 68-kDa BBMV proteins on blots was greatly inhibited by the presence of Cry1Ac, suggesting binding of Cry1Ac to both BBMV glycoproteins. SBA binding was not affected by Cry1Ac mutant ^{509}QNR^{511}-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 glycan moiety 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 (BmAPN1) and cadherin-like protein (BtR175) 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 BmAPN1, suggesting that their binding sites (epitopes) are located close to the BmAPN1 binding site of the toxin. To identify the BmAPN1 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 BmAPN1 binding to the Val582Cys toxin, but not BtR175 binding to it, and reduced the toxicity of Val582Cys toxin in *Bombyx mor*i larvae. These results suggest that the BmAPN1 binding site on Cry1Aa is located near 582Val and that BmAPN1 functions as a receptor for Cry1Aa toxin in *Bombyx mori* larvae. Currently, we are analyzing the BtR175 binding site on Cry1Aa using the same methods.

Poster / Bacteria. B-18.

The chymotrypsin mutants of *Bacillus thuringiensis* Cry1Aa toxins: planar lipid bilayer and light scattering analyses, interaction with *Manduca sexta* midgut receptors

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The *Bacillus thuringiensis* Cry1Aa -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 exhibits its insecticidal activity. This activity is correlated with the toxin's capability to integrate into and form membrane pores that disrupt the ionic balance of target guts epithelial cell 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 -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 larvicidal activity. Additionally, the 65-kDa trypsin-treated Q149P toxin (*ca*. 40nM to 380 nM) was still able to perturb liposome vesicles in calcein-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 -endotoxins is being involved in structural integrity, receptor binding, insect specificity or ion-channel regulation. In this study, the cloned domain III of Cry4B -endotoxin was over-expressed as a soluble form in *Esherichia coli* upon IPTG induction. However, *E.coli* cells expressing the domain III protein showed no toxicity against *Aedes aegypti* mosquito larvae. Circular dichroism spectroscopy indicated that the 23-kDa domain III fragment exists as a -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^{-1} 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 *Photorhabdus luminescens, Xenorhabdus nematophilus* and *Yersinia pestis.* A strain of *Yersinia 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.

STU 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 also have 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 Cry1Ac 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 Phillipines-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.

STU Poster / Bacteria. B-24.

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 Cry1Ac 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) ^{125}I-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, mannose and N-acetylglucosamine, and the lectin wheat germ agglutinin, had little inhibition binding effects for both toxins, in contrast, concavalin A lectin (which binds mannose) strongly inhibited binding of both toxins.

Poster / Bacteria. B-25.

Identification of the western spruce budworm midgut receptor for *Bacillus thuringiensis* insecticidal Cry toxins

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The mode of action of *Bacillus thuringiensis* Cry toxins, which results in cell lysis and larval death, involves proteolytic activation of the protoxins in the lepidopteran gut and 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 Ca^{2+} binding sites that interact with the calcium mimic 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.

Poster / Bacteria. B-26.

***Wolbachia* in sucking lice**

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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.

Poster / Bacteria. B-27.

Molecular evidence and phylogenetic relationships of *Wolbachia* infection in wasps parasitic on pest flies affecting livestock

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Wolbachia, a group of maternally transmitted intracellular parasitic bacteria in invertebrates, has been identified as the culprit for inducing a variety of reproductive disruption and modification in their hosts includeing parasitic wasps. Such changes include feminization of genetic males, male killing, induction of thelytokous parthenogenesis, and cytoplasmic incompatibility. Molecular identification using PCR assays with primers specific for the general *wsp* and *wsp-A* and *-B* genes were conducted to disclose the presence and identity of *Wolbachia* strains/groups in filth fly parasitoids use as biocontrol agents. A total of 53 populations representing 20 species, from 7 countries were tested. Assays revealed over 58% *Wolbachia* infection in this guild of wasps. Among the identified strains, group-A comprised over 85%, none were infected with only B wolbachiae but the rest were doubly infected with A and B wolbachiae. A phylogenetic analysis was done using both DNA sequences from wsp and ftsZ genes. The implications of these infections and phylogenetic relationships of *Wolbachia* in these wasps will be discussed.

STU Poster / Bacteria. B-28.

Adenylyl cyclase and protein kinase A affected the hemocytes-mediated responses of *Malacosoma disstria* to *Xenorhabdus nematophila* and *Bacillus subtilis*

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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 elicit 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 entomopathogenic 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 essentially 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*.

Immuno-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

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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 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* PMFI296. 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.

Poster / Bacteria. B-30.

Endoparasitic nematodes as targets of nematicidal _Bt_ crystal proteins in transgenic plants

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Plant-parasitic nematodes (PPNs) cause an estimated annual economic loss of $100 billion wordewide. 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 presented an environmentally-friendly alternative to chemical pesticides for the control of insect pests. Our laboratory has further confirmed that four Cry proteins (Cry 5B, Cry6A, Cry14A, Cry21A) 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 trouble shoot unknown factors causing 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: interited symbionts with a cut-throat strategy

Michael E. N. Majerus[1] and Helen E. Roy[2]

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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-killing. Within the bacteria, three of these strategies appear on current evidence to be mainly the province of *Wolbachia*. The exception is male-killing 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-killing 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 onthe evolution of host reproductive strategies, including intragenomic conflict, changes in mating preferences, investment in copulation and sex role reversal will be discussed. Areas warranting further investigation will be highlighted.

WEDNESDAY - 30 July

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

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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

modulated. By understanding the mechanisms of disease and parasite resistance of social insects, we may be able to better circumvent such adaptations and develop new biorational control techniques. The use of biological agents to control social insect pests requires acknowledgement that natural selection has favored the evolution of effective means of disease resistance, which could ultimately hinder attempts at biocontrol.

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 environment, the infectious propagules are buffered from detrimental fluctuations 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 termites 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 *Reticulitermis 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 10^8 conidia/g of soil. Amending this assay with sublethal concentrations of selected neonicatinoids caused termites to succumb to mycoses**.** A series of experiments determined that exposure to neonicatinoid 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

Termites are considered as good candidates for control with pathogens 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 to in order to quantify the impact of fungal treatments on field colonies.

Symposium. Wednesday, 9:00.

Microorganisms used for control of fire ants

Roberto M. Pereira, David H. Oi and Dave F. Williams

USDA-ARS, Center for Medical, Agricultural and Veterinary Entomology, 1600 SW 23rd Drive, Gainesville, FL 32608, USA

Imported fire ants (IFA), *Solenopsis* spp., are invasive species that occupy over 320 million acres in the United States causing >6.5 billion dollars per year in damage and impact on the American economy. 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 solutions to the fire ant problem in North America because these pests occur in extensive areas with little economic value where expensive control interventions are prohibitive. The protozoan *Thelohania solenopsae* (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. solenopsae* 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 *Mattesia* protozoan recently described from fire ants in Florida seems to have little direct impact on the ant population. This and other diseases that occur sporadically in fire ant populations may be important factors in maintaining the pest population under stress and therefore less tolerant of other control agents.

Symposium. Wednesday, 9:20.

Managing an unwanted visitor at Acadia National Park

Ellie Groden, Frank Drummond and Shicai Yan

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Myrmica rubra L. is a common species of ant in damp pastures, riverbanks and woodland edges in Europe and Central Asia. Although

locally abundant in these habitats, *M. 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 *M. rubra* at six sites 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. Glare[1], Andrew F. Reeson[2] and Andrew D. Austin[3]

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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 insect pathogens, such as fungi (e.g., *Beauveria, Metarhizium* and *Aspergillus),* entomopathogenic nematodes (*Steinernema* 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 (Div. of Bacteria). Wednes., 8:00-10:00, 2:00-3:00.

Mode of action of bacterial toxins

Symposium. Wednesday, 8:00.

Transgenic Vip Crops for Insect Control

Mikyong Lee, Fred Walters, Hope Hart, Shank Palekar, and Eric Chen

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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 Lepidopteran insect pests including black cutworm (*Agrotis ipsilon*), fall armyworm (*Spodoptera frugiperda*), beet armyworm (*S. exigua*), tobacco budworm (*Heliothis virescens*), corn earworm (*Helicoverpa zea*), 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 prior to cell lysis and larval 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. Characterization 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.

Symposium. Wednesday, 8:30.

The ADP-ribosylating mosquitocidal toxin (MTX) from *Bacillus sphaericus* SSII-1

Jörg Schirmer, Irina Carpusca and Klaus Aktories

Dept. of Experimental and Clinical Pharmacology and Toxicology, Univ. of Freiburg, 79104 Freiburg, Germany

The mosquitocidal toxin (MTX) from *Bacillus sphaericus* SSII-1 is a ~100 kDa protein sharing sequence homology within the N-terminus with the catalytic domains of various bacterial ADP-ribosyltransferases. Chymotrypsin treatment of the 97 kDa MTX holotoxin (MTX^{30-870}), without its putative signal sequence, results in a 70 kDa putative binding component ($MTX^{265-870}$) and a 27 kDa enzyme component (MTX^{30-264}). Proteolytical cleavage is necessary to activate ADP-ribosyltransferase activity of MTX. MTX^{30-264} can also be generated by chymotryptic cleavage of an N-terminal 32 kDa fragment of MTX (MTX^{30-308}) but ADP-ribosyltransferase activity is largely enhanced compared to the processed holotoxin. Precipitation analysis shows that the 70 kDa proteolytic fragment of MTX remains non-covalently bound to the N-terminal 27 kDa fragment and thereby inhibits enzyme activity. Kinetic data suggest that the MTX binding component acts like a non-competitive inhibitor on MTX enzyme activity. We were able to show that the first 21 amino acids of the putative MTX binding component are sufficient to elicit an inhibitory effect on the MTX enzyme component. Changing the acidic character of this peptide $MTX^{265-285}$ by exchanging aspartat residues to asparagin residues markedly decreased its enzyme activity inhibiting abilities. However, the inhibitory effect of the peptide $MTX^{265-285}$ is reduced compared to the complete 70 kDa MTX fragment presumably due to a lower affinity to the MTX enzyme component.

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 Schwartz[1,2], Adelaida Maria Gaviria Rivera[3], Léna Potvin[1], Colin Berry[3] and Gianfranco Menestrina[4]

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B. sphaericus (*Bs*) is a spore-forming bacterium that produces several mosquitocidal toxins: high toxicity strains express the binary toxins (Bin toxins: BinA, a 42k-Da 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 toxins includes a 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 10µg/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 observable current steps and plotting it against applied voltage. P70 conductance was approximately 600 pS. FTIR spectroscopy indicated that P70 was made of 17% α-helices and 63% β-structures. Our data suggest that the 70-kDa C-terminal fragment of *Bs* Mtx toxins permeabilizes cell membranes by the formation of pores.

Symposium. Wednesday, 9:30.

Biochemical and biophysical properties of PS149B1, a binary toxin from *Bacillus thuringiensis*

Luke Masson[1], George Schwab[2] and Jean-Louis Schwartz[1]

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B. thuringiensis PS149B1 binary toxin is made of two components, Cry34Ab1 (14-kDa) and Cry35Ab1 (44-kDa), which, combined, are toxic to the Western corn rootworm. The behaviour of the native binary toxin and its two individual re-combinant components was studied using a variety of biochemical and biophysical techniques. The 44-kDa component and the PS149B1 crystal were completely digested by papain, whereas the 44-kDa protein was sequentially degraded by trypsin to a 40-kDa and smaller fragments. Chymotrypsin also displayed a sequential degradation with an extremely rapid activation producing a 40-kDa fragment. Renografin selectively solubilized the 44-kDa component over the 40-kDa fragment. The conversion of the 44-kDa moiety into the 40-kDa fragment was pH-dependent with maximum conversion at pH 5 to 6. Blue native gels showed that the two components of PS149B1 did not interact strongly, although weak interaction was observed in surface plasmon resonance studies. The 14-kDa component was present as monomer in solution and the purified 40-kDa fragment could be isolated primarily as a monomer, whereas the 44-kDa component always formed large aggregates. The PS149B1 binary, its individual components, the 40-kDa fragment and the stoichiometric mixtures of the 14-kDa component with either the 44-kDa component or the 40-kDa fragment all formed ion channels in planar lipid bilayers. Well resolved current steps were always observed on top of baseline currents suggesting either that some channels remained locked in the open state or that the membrane became leaky when exposed to the proteins. Activity was characterized by long channel openings. The conductances of PS149B1 ranged between 310 and 920 pS (up to 2.7 nS) and those of the 14-kDa + 40-kDa mixture between 20 and 765 pS. Both the toxin and the mixture integrated the membrane easily (62% and 33% success rates, respectively). The 14-kDa + 44-kDa mixture (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**

T. Hey, S. Bevan, A. Schleper, P. Birkhold, S. Russell, R. Thompson, J. Sheets, Z.-S. Li, J. Lira, S. Bintrim, K. Fencil, W. Ni, D. Merlo and T. Meade

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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 bassicae*, *Pieris rapae*, *Plutella xylostella*, *Heliothis virescens, H. zea*, and *Phaedon cochleariae*; 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. bovienii* 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 (*xpt*A, *xpt*B and *xpt*C - 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 *Xenorhabdus* 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 nematicidal activity identified in one strain has been charcterised and requires two proteins, *xnp*1 and *xnp*2. 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

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 Isobe[1],Takahiro Matsuyama[1], Katura Kojima[1,2], Toshio Kanda[2], Toshiki Tamura[2], Ken Sahara[1], Shin-ichiro Asano[1] and Hisanori Bando[1]

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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 silkworm 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 hrs 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 *pIE*1-4 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 *Sf9* cells are infected with AcMNPV lacking p35 gene (AcMNPVΔP35), but AcMNPVΔP35 virus replication and occlusion body formation could be rescued when cells were transfected with *pIE*1-4*Amiap*.

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 ATI 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 *Ld*652 cells compared to the parental virus, the *iap* knockout virus can be propagated in *Ld*652 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 *SL*2 cells, and the ability of the *iap* knockout virus to form plaques was significantly impaired compared to parental virus in semi-permissive *Sf*9 cells. Caspase-3 activity, an indicator of apoptosis, was also significantly increased in *Ld*652, *Sf*9 and *SL*2 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.

Pariacoto nodavirus wild-type virus particles contain a minor protein translated from the second AUG codon of the capsid open reading frame

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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 *Pariacoto 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 encapsidates 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 ACG. 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 M25I 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 M25I 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)

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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 entomopoxvirus. Superoxide dismutases (SOD) catalyze the conversion of superoxide radicals (O_2^-

) 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 Cu^{2+} and Zn^{2+}. The recombinant protein contains a 6X-His N-terminal epitope tag and the protein was purified using Ni^{++} affinity chromatography. The resulting protein was determined to contain 42% Cu and 115% Zn. This recombinant protein, unlike vertebrate poxvirus SODs, is active in both an *in situ* gel assay of electrophoretically separated proteins and as determined by stopped flow spectrophotometry. The k_{cat}/K_m 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 late in infection. Furthermore we have demonstrated using 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 the DsRed2 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

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RING finger proteins mediate diverse cellular processes, e.g., oncogenesis, apoptosis, development, viral infection, transcriptional repression and ubiquitination. AcMNPV *exon0* ORF is 786 bp and codes for predicted 261 aa protein. This protein contains a novel RING finger motif (C_3YC_4) 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 C_3FC_4. 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 of GP64 membrane localization

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The major envelope glycoprotein (GP64) of the virus AcMNPV, is post-translationally modified by palmitoylation. By ^{3}H-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 *gp64* 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 viruses expressing palmitoylation-minus forms of 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 and 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*

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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 documented. 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 baculovirus envelope fusion protein genes 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-cF. Using vectors that would generate Dm-cF-GFP fusion protein and Dm-cF-V5 epitope tagged protein, we showed that unlike viral F proteins which localize to the plasma membrane when transiently expressed in cultured cells, Dm-cF transiently expressed in DmS2 cells localizes to discreet cytosolic compartments within the cytosol. In addition, our RT-PCR results suggest that Dm-cF expression is developmentally-regulated. Dm-cFexpression was first detected in 3rd instar larvae, but was also detected in the pupal stages and in adults of both sexes. Analysis of Dm-cF 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

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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 disivion 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 post-translationally cleaved by a cellular proprotein convertase (e.g. furin) into two disulphide-linked subunits (F_1 and F_2). 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 gp64null 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 alphahelical 'fusion domain' with a hydrophobic side which can then interact with host cell membranes. The N-terminus of F_1 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

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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*

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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

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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

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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.

SYMPOSIUM (Div. of Bacteria). Wednes., 3:00-4:00, 4:30-6:30.

Mode of action of three-domain Cry toxin family

Symposium. Wednesday, 3:00.

Mapping Binding Epitopes on Cry Proteins

Mohd Amir F. Abdullah[1], Autumn White[1], Rebecca J. McNall[2], Michael J. Adang[2] and Donald H. Dean[1]

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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 *Cx. pipiens* 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 Zhuang[1,2], Ruiyu Xie[1,2], Isabel Gomez[3], Mario Soberón[3], Alejandra Bravo[3], Linda S. Ross[2] and Sarjeet S. Gill[1,2]

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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 Cry1Ac resistant *H. virescens* strain (Gahan et al., 2002. Science), while suppression of aminopeptidases in vivo decreases Cry1C toxicity (Rajagopal et al., 2002). Consequently both receptors apparently are required for toxicity of Cry1 toxins, and to understand the role of these two receptors in toxin action we evaluated their role in Cry1A toxin interaction in *H. virescens* membrane preparations. Our laboratories have hypothesized that microdomains or lipid rafts play a role by which these two receptors play a role in toxicity. To understand the potential role of lipid rafts in toxicity we isolated and analyzed the protein and lipid components of these lipid raft microdomains from the midgut epithelial membrane of lepidopteran insects. We showed that like their mammalian counterparts, *H. virescens* and *M. sexta* lipid rafts are enriched in cholesterol, sphingolipids, and GPI-anchored proteins (Zhuang et al., 2002). We showed that several putative *Bacillus thuringiensis* Cry1A receptors, including the 120- and 170-kDa aminopeptidases from *H. virescens*, the 120-kDa aminopeptidase from *M. sexta*, were preferentially partitioned into lipid rafts. We also demonstrated that Cry1A toxins were associated with lipid rafts, and that lipid raft integrity was essential for *in vitro* Cry1Ab pore-forming activity. Our study strongly suggests that these microdomains might be involved in Cry1A toxin aggregation and pore formation. More recently we have begun analyzing the interaction of the cadherin with these lipid rafts. Under normal conditions the cadherin receptor is not associated with rafts. However, with addition of toxin, there is a change in the distribution of this receptor. The implications of these changes in receptor association with respect to toxicity will be discussed. **References:** Gahan, L. J., Gould, F., and Heckle, D. G. (2001) *Science* 293, 857-860. Rajagopal, R., Sivakumar, S., Agrawal, N., Malhotra, P., and Bhatnagar, R. K. (2002) *J. Biol Chem* 107, 1619. Zhuang, M., Oltean, D. I., Gomez, I., Pullikuth, A. K., Soberon, M., Bravo, A., and Gill, S. S. (2002) *J Biol Chem* 277, 13863-13872.

Symposium. Wednesday, 2:00.

Interaction of Cry1A toxin with BtR1 and its role in a pre-pore formation

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In susceptible lepidopteran insects aminopeptidase-N (APN) and cadherin-like proteins (Bt-R_1) are the putative receptors for Cry1A toxins. The binding affinity of APN is on the range of 100 nM while that of Bt-R_1 is on the range of 1 nM. The differences of binding affinities between APN and Bt-R_1 suggest that binding to Bt-R_1 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-R_1 receptor. Incubation of Cry1Ab protoxin with two Bt-R_1 peptides of 70 residues, corresponding to the two toxin binding regions, or with a single chain antibody scFv73 that mimics Bt-R_1 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 Vachon[1,2], Raynald Laprade[1,2], Jean-Louis Schwartz[1,2,3] and Luke Masson[2,3]

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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

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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: aminopeptidases 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 transfected cells. 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 Bt-R1 cadherin were less surprising as Cry1A toxin binding was positively correlated with S2 cytotoxicity. Truncated forms of Bt-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 transfected cells, and PI established cytotoxicity. This system holds promise for future investigations of Cry toxin action.

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

VIRUSES – 5

Contributed paper. Wednesday, 2:00.

A persistent baculovirus infection in a laboratory-reared culture of *Trichoplusia ni*?

Richard Hitchman[1,2], John P. Burden, Linda A. King[2], Rosie S. Hails[1] and Robert D. Possee[1]

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Baculoviruses are best known for the lethal infections they induce in susceptible insect larvae. The virus-infected host typically liquefies after death and releases large quantities of occlusion bodies with the potential to continue the replication cycle in a new insect. If a host is not immediately available, dogma has it that the virus can survive in the environment for extended periods- probably years. However, persistent virus infections have also been identified in populations of *Mamestra brassicae* reared in the laboratory and those recently collected in the field. In contrast with sublethal baculovirus infections of insects, the host appears to incur little cost by carrying these persistent infections. Recent studies have shown that the results with *M. brassicae* are not unique. *Trichoplusia ni* is a host commonly used to propagate a number of baculoviruses, including *Autographa californica* nucleopolyhedrovirus (AcNPV). We used it to analyse replication of *Panolis flammea* (Pf) NPV in an alternative host. Subsequent purification of virus DNA from polyhedra derived from PfNPV-challenged *T. ni* revealed the progeny virus was unrelated to the inoculum. Further analysis using restriction enzymes suggested that the virus amplified in *T. ni* was most closely related to AcNPV or TnNPV, although significant differences in fragment sizes were evident. When the virus DNA was used to transfect *Spodoptera frugiperda* cells it induced typical signs of baculovirus infection *in vitro*, culminating in production of polyhedra very late in infection. Direct evidence for the presence of a persistent baculovirus infection in *T. ni* was provided by using reverse transcription coupled with the polymerase chain reaction to detect polyhedrin-specific transcripts present in mRNA prepared from healthy larvae. The DNA products from these experiments were sequenced and aligned precisely with AcNPV polyhedrin gene sequences. These data suggest that a laboratory maintained culture of *T. ni* habours a persistent baculovirus closely related to AcNPV or TnNPV. It remains to be seen whether or not field isolates of this species also have a persistent virus infection.

Contributed paper. Wednesday, 2:15.

Molecular mechanism for HaNPV transporting to the host nucleus

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Heliothis armigera nuclear polyhedrosis virus (HaNPV) is the earliest commercial baculovirus insecticide to prevent *Heliothis armigera* in China. Using the major capsid protein of HaNPV, VP39, as bait protein, the cellular interactional factor Actin was isolated from the cDNA library of Hz-AM1 cells through yeast two-hybrid system. The purified VP39 and Actin could combine and form hybrid-band in the Western blot experiment. The combining constant for such two proteins measured by Isothermal Titration Calorimeter technology is 10^5 and the ΔH is 2.871E7. Under the control of the AcMNPV IE1 promoter, the expression of the fusion protein GFP-VP39 coud induce the actin cytoskeleton to form cable struture in Hz-AM1 cells. Using cytochalasin D (CD) to prevent global actin from forming filamentous structures, HaNPV formed incomplete virions but the host actin concentration and the replication of viral DNA was not influenced. The transportation of HaNPV nucleocapsid from the cytoplasm to the nucleus was inhibited by the pretreatment of the host cells with cytochalasin D; while colchicines had not such effect. This result demonstrated that the transportation of HaNPV nucleocapsid from the cytoplasm to the nucleus was associated with Actin filament but not microtube. The infection of the recombinant virus

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

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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 braconid *Glyptapanteles indiensis* PDV (GiPDV). Junction clones containing both insect chromosomal and polydnaviral 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 retrotransposon, 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 vitro* integration.

Contributed paper. Wednesday, 2:45.

A phage-displayed peptide can inhibit infection of white spot syndrome virus of shrimp

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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 K_{aff} is 7.28 x 10^{-9}) and appeared capable of inhibiting virus infection completely at a peptide concentration of about 400 nmol 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 LT_{50} (more than 20 days). The sequencing results showed the possible critical motif for viral inhibition being **VAV**NN**S**Y. 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

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Tetraviruses are small, positive-sense RNA viruses that specifically infect Lepidopteran insects. The two genera within the *Tetraviridae* 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 zea* 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 omegatetraviruses. 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 *Thosea asigna virus* (TaV) and Euprosterna elaeasa 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 *Tetraviridae*.

Contributed paper. Wednesday, 3:15.

Baculovirus diversity: Establishment of a natural classification system using molecular phylogeny

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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, lef-8, lef-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.

Contributed paper. Wednesday, 3:30.

BUGs: The Baculovirus Updated Genome site enabling *virologists* to keep pace with genome sequencing

Sarah L. Turner, Milo Thurston, Robert D. Possee and Dawn Field

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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 (syntenies) 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 Arif[4], Hilary A.M. Lauzon[1], Christopher Lucarotti[2] and Peter Krell[3]

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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 residues content (67%) indicating a bias of codon usage. Most of the genes associated with transcription, DNA replication and those encoding viral structural proteins were identified in the NeleNPV genome. However, most of the auxiliary genes, the typical baculoviral homologous repeated sequences (*hrs*) and the bro genes were absent. Notable is the absence of a membrane fusion protein (F-protein) present is all NPV genomes reported so far which raised the question on the presence of the extra cellular virus (ECV) phenotype in the replicative cycle. Since tissue tropism of NeleNPV is restricted to the midgut, this virus may not require the ECV phenotype. The small genome size reveals that the total number of genes conserved among all baculoviruses sequenced so far is now 29. Phylogenetic analysis based on all the 29 conserved genes show that the NeleNPV and the *Culex nigripalpus* NPV are out groups and do not share a clade with the other NPVs or GVs.

Symposium. Tuesday, 4:58.

Sequence analysis of the genome of *Neodiprion sertifer* single-nucleocapsid nucleopolyhedrovirus

James E. Maruniak, Alejandra Garcia-Maruniak, Aissa Doumbouya, Tom Merritt, Jaw-Ching Liu, Jennifer Lanoie and Ronit Kesari

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Research on the lepidopteran baculoviruses has identified core genes that are conserved amongst their genomes. Since the hymenopteran baculoviruses are phylogenetically more ancestral than the lepidopteran baculoviruses, a comparison of the hymenopteran, dipteran and lepidopteran baculovirus genes would give a better picture of the essential core genes of the family Baculoviridae. A total of 19 baculoviruses genomes have been completely sequenced and are available in GenBank. From these all but one belong to baculoviruses capable of infecting lepidopteran insects. Currently the genomes of two hymenopteran baculoviruses have been completed and will be added to the previously mentioned list. This work presents the data from the *Neodiprion sertifer* nucleopolyhedrovirus (NeseSNPV), a hymenopteran baculovirus pathogenic to the European pine sawfly in forest commodities in Europe and North America.

The genome of NeseSNPV is 86,462 bp. The NeseSNPV has low DNA sequence homology compared to the lepidopteran NPVs. A total of 89 ORFs with more than 50 amino acid and minimum overlapping were found. The nucleotide composition is 66% A+T making it an AT rich genome. The transcriptional orientation of the ORFs is higher clockwise with 54 ORF (60.7%) than counterclockwise with 35 ORFs (39.3%). BLAST analysis of NeseSNPVgave a total of 43 ORFs homologous to other baculoviruses under the criteria used for the analysis. The aa identity found between NeseSNPV and the lepidopteran baculoviruses was very low. This made the determination of gene identity difficult by BLAST searching. Some genes that have been considered essential baculovirus core genes were not found in NeseSNPV.

Symposium. Tuesday, 5:18.

Analysis of molecular adaptation of nucleopolyhedrovirus genes

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Genes in which nonsynonymous substitutions are fixed at a higher rate than synonymous substitutions are said to have undergone adaptive molecular evolution, or 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*, *ac75*, *ac103, dnahel*, *dnapol*, *lef-10*, *lef-12*, *odv-e18*, *odv-e56*, *p6.9*, *pk1*, *vp39*, and *vp80*. NPV genes that have undergone positive selection may modulate the ability of different NPVs to replicate efficiently in cells (*dnapol*, *dnahel*, *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. Herniou[1,2], Julie Olzewski[1], David O'Reilly[3] and Jenny Cory[2]

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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

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)

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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.

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

FUNGI – 3

Contributed paper. Tuesday, 4:30.

Phylogeography of the insect pathogenic fungus, *Metarhizium*

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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.*

Contributed paper. Tuesday, 4:45.

Molecular mechanisms of adaptive radiation in *Metarhizium anisopliae*

Raymond St. Leger and Gang Hu

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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 representing 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 states.

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.

Contributed paper. Tuesday, 5:00.

A multigene phylogeny of *Beauveria*: new insights into species diversity, biogeography, host affiliation and life history

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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. caledonica* 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. caledonica* 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. staphylinidaecola* 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

Contributed paper. Tuesday, 5:15.

Phylogenetic and population genetic approaches to the analysis of cryptic speciation in the *Beauveria bassiana* s.str. complex

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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

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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 of and 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*

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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 (LC_{50} values <100 spores/mm^2 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/mm^2) 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/mm^2) 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

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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 Sabouraud dextrose agar amended with 1% yeast extract at 27ºC with a 12h photophase and harvested 12 days after inoculation. Conidia were then transferred and kept in an Auto-desiccator Cabinet (Scienceware®, Pequannock, NJ, USA). The loss of water in the drying chamber was stabilized at 104h drying. The contents of water (dry weight) of fresh and dried conidia were 66 and 1%. Conidia were kept in the drying chamber for 7 days to assure they had been dried

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

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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 (2.1×10^8 blastospores/cm^2 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 are 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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