2011 International Congress on Invertebrate Pathology and Microbial Control &
44th Annual Meeting of the Society for Invertebrate Pathology

ABSTRACTS

07-11 August 2011

Saint Mary's University
Halifax, Nova Scotia
Canada

MONDAY – 8 August

Plenary Symposium Monday, 10:30-12:30

Disease Perspectives from the Global Crustacean Fishery

Plenary Symposium, Monday 10:30 **1**

Crustacean diseases – A Canadian perspective

Rick Cawthorn
Department of Pathology and Microbiology, AVCLSC

Plenary Symposium, Monday 11:00 **2**

Crustacean diseases – A US perspective

Jeffrey D. Shields
Virginia Institute of Marine Science, The College of William and Mary, Gloucester Point, VA 23062, USA
Address for correspondence: jeff@vims.edu

Commercial industries for crustaceans in the USA have two primary modes of production: aquaculture and fisheries. Crayfish and penaeid shrimp support crustacean aquaculture industries in America. The disease issues in aquaculture arise from intensive culture conditions, such as high stocking density, the import of contaminated stock, poor water quality, and the lack of suitable quarantine control. Microbial pathogens are of significant concern in these settings, most notably being viruses in penaeid shrimp. White spot syndrome virus (WSSV), taura virus, infectious hypodermal and hematopoietic necrotizing virus, and yellowhead virus are just a few of the more than 20 pathogenic viruses reported from penaeid shrimp. Several of these have been introduced into the USA causing considerable damage to the nacent culture industry.

A more diverse group of crustaceans support commercial fisheries, including a variety of crabs (blue, Dungeness, rock, snow, and king crabs), penaeid shrimps, lobsters (clawed and spiny), as well as those harvested for use in aquaculture production such as brine shrimp. The disease issues in fisheries are often associated with environmental factors such as entrained water masses, increased climatic variability and seasonal molting, or factors directly associated with the fishery such as removal of adults or males, and large-scale changes to host biomass. Outbreaks of pathogens have damaged several important fisheries, but the causative agents were often unknown at the time. Nonetheless, the pathogens are as diverse as their hosts, and range from microbial agents associated with epizootic shell disease on clawed lobsters, parasitic dinoflagellates in blue crabs and snow crabs, and nemertean egg predators on rock and king crabs, to rhizocephalan barnacles in blue and king crabs. In aquaculture and in fisheries, direct effects such as dead shrimps or crabs are obvious, but less obvious are the morbid effects of disease which include enhanced susceptibility to other diseases, increased predation risk, loss of vigor, stunting, and parasitic castration.

Plenary Symposium, Monday 11:30 **3**

Crustacean Diseases – an EU Perspective

Stentiford, G.D.
European Union Reference Laboratory for Crustacean Diseases, Centre for Environment, Fisheries and Aquaculture Science (Cefas), Weymouth Laboratory, Weymouth, Dorset DT4 8UB, United Kingdom
Address for correspondence: grant.stentiford@cefas.co.uk

Crustacean production in European marine waters is based around a large cold-water fishery for crabs, lobsters and shrimp and a small aquaculture industry for temperate water shrimp and crayfish. Despite the commercial and ecological importance of these populations, studies on their diseases are a relative deficit discipline compared to those from molluscan and finfish host groups. In addition to capture production from native stocks, European states are major importers of farmed crustaceans (mainly tropical shrimp) as these products become an increasingly significant component of the European seafood diet. Due to these factors, EC Directive 2006/88, applied from August 2008, has for the first time listed the three viral diseases White Spot Disease (WSD), Yellowhead Disease (YHD) and Taura Syndrome (TS) as exotic pathogens of concern. In addition to the listing of these pathogens, and in line with infrastructural arrangements for fish and mollusc diseases, the EC have designated a European Union Reference Laboratory (EURL) to cover crustacean diseases, with individual Member State National References Laboratories (NRL) being designated by Member State Competent Authorities. The designation of an EURL for crustacean diseases formally recognizes the ecological and commercial importance of crustaceans in the aquatic habitats of EU Member States and also the potential for exotic disease introductions to these populations via the international trade of live and commodity products. The designations have also revealed a relative paucity in crustacean disase expertise across the EU. Improvements in the biosecurity status of native crustacean populations within the EU and a concomitant enhancement of knowledge on native pathogens and mortality drivers in natural and fared stocks is expected to develop in coming years. This presentation will discuss the crustacean disease components of EC Directive 2006/88, place this into context for nations importing live crustaceans and their products to the EU and highlight current research interests of the EURL.

Plenary Symposium, Monday 12:00 **4**

Crustacean diseases - an Australian perspective

Brian Jones
Department of Fisheries, Government of Western Australia, P.O. Box 20, North Beach, WA 6920, Australia
Address for correspondence: brian.jones@fish.wa.gov.au

In Australia, crustacean fisheries are based on trawling for shrimps ("prawns") as well as potting for panulirid lobsters, *Jasus* spp. and *Panulirus* spp. with small incidental catches of Bay lobsters *(Thenus* spp.), nephrops lobsters and crabs; as well as aquaculture of freshwater crayfish (*Cherax* spp.). 57% of the gross value of production of Australian fisheries comes from crustacean landings, of which rock lobsters form 18% and shrimp 13% (2008-09 exports for Australia worth about US$437 000 000 while shrimp were valued at $312 000 000).

Investigation of disease has been patchy. This is due, in part, to the absence of significant aquaculture, except for shrimp; the lack of disease diagnostic expertise generally and finally to the difficulty in sampling sick animals from wild fisheries using nonrandom sampling methods. The inability to adequately sample animals in the open ocean has been a problem, for example, during recent low recruitment to the western rock lobster fishery.

Though Australasia remains free of White Spot Syndrome Virus (WSSV), virus diseases have been a problem in the shrimp aquaculture industry. A recent (2009) Import Risk Assessment (IRA) determined that WSSV, taura syndrome virus and yellowhead disease posed an unacceptable risk to Australia. The IRA process highlighted three problems: The lack of information on disease, including variant strains; the very high cost of the IRA process, and the tension that exists in the community between "free trade" and protection of native biodiversity.

Student Workshop Monday, 12:30-14:00

How to Write Grant Proposals

Organizers: Sastia Putri and Kelly Bateman

Student Workshop, Monday 12:40 **5**

How to approach senior scientists for joint grant writing

Jørgen Eilenberg

Student Workshop, Monday 13:00 **6**

Making sure your grant is funded

Ann E. Hajek
Department of Entomology, Comstock Hall, Cornell University, Ithaca, NY 14853-0901
Address for correspondence: aeh4@cornell.edu

When you spend the time writing a grant proposal you want to maximize the chances that reviewers will give your grant top marks so that it is funded. We'll discuss how to organize and write your grant proposal and making sure to get pre-submission reviews and, in particular, making sure to allocate enough time.

Student Workshop, Monday 13:20 **7**

A winning proposal: effective grant writing for early career researchers

Helen Hesketh
Centre for Ecology and Hydrology, Maclean Building, Benson Lane, Crowmarsh Gifford, Wallingford, Oxfordshire, OX10 8BB, United Kingdom
Address for correspondence: hhesketh@ceh.ac.uk

Writing a proposal to secure research funds is a daunting and time consuming process. However, with forward planning and good preparation it can also be an enjoyable and rewarding experience. No matter which organisation your proposal is directed to and what your topic is, every proposal reader is looking for clear answers to key questions such as: what are we going to learn as a result of this work that we don't already know, why is it worth knowing and how will we know the conclusions are valid? A good proposal will have a number of common elements that make this easy for an assessor. The main three elements that a peer review panel will consider are: importance and impact, novelty of approach and sound methodology. A good proposal will be based on an outstanding scientific question which is addressed through simple, clear hypotheses. These hypotheses should be tested using sound methodology which produces validated results. However, the infrastructure of a proposal should be taken as seriously as the science itself and supporting materials such as budgets should be carefully considered. I will provide examples of good practice and potential pitfalls, based on experience of CEH (Centre for Ecology & Hydrology) staff in making UK and EU grant applications. There are many grants available specifically for early career researchers so it is worth becoming familiar with writing a good grant proposal and requirements of potential funding bodies in order to take advantage of these funds.

Contributed Papers Monday, 14:00-16:00

Bacteria 1

Contributed Paper, Monday 14:00 **8**

Identification of hemipteran-active *Bacillus thuringiensis* crystal proteins and evaluation of *in planta* efficacy for control of Lygus bugs in cotton

James A. Baum, Uma R. Sukuru, Stephen R. Penn, Steven E. Meyer, Shubha Subbarao, Xiaohong Shi, Stanislaw Flasinski, Gregory R. Heck, Robert S. Brown, and Thomas L. Clark
Monsanto Company, 700 Chesterfield Parkway West, Chesterfield, Missouri 63017
Address for correspondence: james.a.baum@monsanto.com

The plant bugs *Lygus hesperus* and *Lygus lineolaris* have emerged as economic pests of cotton in the United States. These hemipteran species are not controlled by the lepidopteran-specific insect control traits (*Bacillus thuringiensis* Cry proteins) found in genetically-modified commercial varieties of cotton. Indeed, there has been no compelling evidence in the scientific literature to indicate that Bt insecticidal proteins can be effective in controlling piercing-sucking insects such as Lygus. We summarize recent work on the identification of novel Bt Cry proteins toxic to Lygus nymphs. Cotton plants expressing a novel 35 kDa crystal protein were observed to impact the survival and development of *Lygus hesperus* nymphs in a concentration-dependent manner. These results provide our first proof-of-concept for the development of cotton varieties protected from Lygus feeding damage.

Contributed Paper, Monday 14:15 **9**

Novel approach for Bt toxin-based transgenic aphid resistance

Nanasaheb P. Chougule, Huarong Li[1], Sijun Liu, Bryony C. Bonning
Department of Entomology, Iowa State University, Ames, IA 50011, USA
[1]Current affiliation, Dow AgroSciences, Indianapolis, IN 46268, USA
Address for correspondence: bbonning@iastate.edu

Although toxins derived from the bacterium *Bacillus thuringiensis* (Bt) have been used successfully for management of some insect pests, Bt toxins are not effective against the agriculturally important Hemiptera. These sap-sucking insects currently present one of the biggest challenges for agricultural pest management, causing both direct damage and serving as vectors for plant disease. Through systematic analysis of the interaction of representative Bt crystal (Cry) toxins with the aphid gut, we established that there are multiple physiological parameters that may account for the lack of Cry toxin activity against aphids. Mutagenesis of Bt toxins can be used to artificially alter toxins for certain properties for enhanced toxicity and we have identified a novel strategy to increase toxin action against aphids. This approach shows promise for production of aphid resistant transgenic plants.

Contributed Paper, Monday 14:30 **10**

A novel transcriptional mechanism of *Bacillus thuringiensis cry8Ea1* toxic to Scarab beetle

Lixin Du[1, 2], Lili Qiu[1], Jie Zhang[1], Shuliang Feng[2], Fuping Song[1*], Dafang Huang[3*]
[1]State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, CAAS, China, [2]Institute of Plant Protection, Hebei Academy of Agricultural and Forestry and Sciences, China, [3]Biotechnology Research Institute, CAAS, China
Address for correspondence: dfhuang@mail.caas.net.cn

Bacillus thuringiensis (*B. thuringiensis*) is an insect pathogen mainly due to production of parasporal crystal toxins encoded by *cry* or *cyt* genes. Transcription and regulation of *cry* genes were well studied. Here we reported a novel transcriptional model for a holotype toxin, Cry8Ea1, toxic to the underground pest *Holotrichia parallela*. RT-PCR results indicated that *cry8Ea1* and its upstream gene, *orf1*, which showed sequence similarity to the first ORF of *cry2A*, *cry9Ec*, *cry9Ca*, *cry11A*, and *cry18A* operons were co-transcripted. However β-galactosidase assay demonstrated that the intergenic region between *orf1* and *cry8E* existed a promoter, named as Pcry8E with transcriptional activity, besides Porf1 promoter, the upstream region of *orf1*. Among other similar *cry* operons, this is a first report that intergenic region

between *orf1* and *cry* gene existed a promoter. Beta-galactosidase assay showed that transcriptional activity of Porf1 was abolished in the *sigE* mutant while transcriptional activity of Pcry8E was significantly decreased in the *sigH* mutant. Transcriptional start sites directed by Porf1 and Pcry8E were determined by 5′-smarter race method respectively. The -35 and -10 regions of Porf1 and Pcry8E showed highly sequence similarity with σ^E and σ^H recognized motif. These results indicated that Porf1 was controlled by σ^E factor and Pcry8E was controlled by σ^H factor.

Contributed Paper, Monday 14:45 **11**

Cyt1A of *Bacillus thuringiensis* restores *Bacillus sphaericus* Bin toxicity via large microvillar lesions, not cation channels, in Bin-Resistant larvae of *Culex quinquefasciatus*

Brian A. Federici [1,2]; Margaret C. Wirth[1]; Jeffrey J. Johnson[1]; Hyun-Woo Park[1]; Dennis K. Bideshi[1]; William E. Walton[1]
Department of Entomology[1] and Interdepartmental Graduate Program Microbiology[2], University of California - Riverside, Riverside, California 92521 USA
Address for correspondence: federici@ucr.edu

The Cyt1A protein of *Bacillus thuringiensis* masks resistance to the *Bacillus sphaericus* Binary (Bin) toxin in Bin-resistant larvae of *Culex quinquefasciatus* and extends its spectrum of activity to *Aedes aegypti*. The mechanism underlying these important properties is unknown. Using purified toxins labeled with fluorescent dyes, we show that sublethal concentrations of Cyt1A enabled the *B. sphaericus* Bin toxin to cross the microvillar membrane to the cytoplasm in Bin-resistant and insensitive larvae, both of which lack Bin's α-glucosidase receptor. In sensitive larvae of *Cx. quinquefascaitus*, Bin exhibited intratissue specificity, whether fed alone or in combination with Cyt1A, binding preferentially to cells in the gastric caeca and posterior stomach. When fed alone to Bin-resistant larvae of this species, or larvae of *Ae. aegypti*, no Bin was observed bound to any region of the midgut epithelium. When fed together, however, within 15 minutes Bin localized in the cytoplasm of midgut epithelial cells, without binding to the microvillar membrane, and along the entire midgut epithelium, whereas most Cyt1A remained bound to microvilli. Toxin binding patterns indicated that Bin did not bind to Cyt1A at any point during intoxication. We conclude that Cyt1A synergizes Bin by creating large transmembrane microvillar lesions of at least 9 nm, a conservative estimate of Bin's minimal diameter, which is much larger than classic cation channels. No cell hypertrophy or lysis, or larval mortality was observed after exposure to Cyt1A for 24 hours, indicating that midgut cells *in vivo* remained viable after creation of these large microvillar membrane lesions.

Contributed Paper, Monday 15:00 **12**

The 60-kDa protein encoded by *orf2* in the *cry19A* operon of *Bacillus thuringiensis* subsp. *jegathesan* functions like a C-terminus of the 135-kDa Class Cry proteins

J. Eleazar Barboza-Corona[1]; Hyun-Woo Park[2,3,4]; Dennis K. Bideshi[2,3]; Brian A. Federici[2,5]
[1]Universidad de Guanajuato Campus Irapuato-Salamanca, División Ciencias de la Vida, Departamento de Ingeniería en Alimentos, Irapuato, Guanajuato, 36500, México, [2]Department of Entomology, University of California, Riverside, Riverside, CA 92521, USA, [3]Department of Natural and Mathematical Sciences, California Baptist University, Riverside, CA 92504, USA, [4]John A. Mulrennan, Sr., Public Health Entomology Research & Education Center, Florida A&M University, Panama City, FL 32405, USA, [5]Interdepartmental Graduate Programs in Genetics, Genomics & Bioinformatics, and Cell, Molecular & Developmental Biology, University of California, Riverside, Riverside, CA 92521, USA.
Address for correspondence: brian.federici@ucr.edu

The *cry19A* operon of *Bacillus thuringiensis* subsp. *jegathesan* encodes two proteins, Cry19A, a 75-kDa mosquitocidal protein (ORF1), and a 60-kDa protein of unknown function (ORF2), the genes for which are separated by a short intergenic sequence. Expression of this operon in an acrystalliferous *B. thuringiensis* strain yielded a small crystal in each cell, with the shape varying from one cell to another, whereas no crystals were produced when either gene was expressed alone. Expression of the *cry19A* operon under control of *cyt1A* promoters combined with the 5' STAB-SD transcript stabilizing sequence increased crystal size four-fold. The larger crystals produced by this construct had a shape similar to classic Cry1 bipyramidal crystals, and contained both the Cry19A and 60-kDa ORF2 proteins. To analyze the function of ORF2, different combinations of Cry19A, ORF2 and the N- or C-terminus of Cry1C were synthesized in *B. thuringiensis*. The *cry19A-orf2* fusion without the intergenic space yielded a crystal similar in size and shape to those produced by the wild type operon. Fusion constructs of either *cry19A* and the C-terminus of *cry1C* or the *cry1C* N-terminus and *orf2* also produced crystals similar to those produced by the wild type operon. However, a N-terminus *cry1C* and *orf2* construct containing the intergenic sequence did not produce a crystal. These results suggest that the 60-kDa ORF2 protein assisted synthesis and crystallization of Cry19A, and thus had a function similar to that of the C-terminal half of 135-kDa class Cry proteins.

Contributed Paper, Monday 15:15 **13 STU**

Using multi-omics approaches to elucidate the bases of resistance to *Bti* in *Aedes aegypti*

Guillaume Tetreau[1], Margot Paris[1], Krishnareddy Bayyareddy[2], Christopher Johnson[3], Michael J. Adang[2,4], Jean-Philippe David[1], Laurence Després[1]
[1]Laboratoire d'Ecologie Alpine, CNRS-UMR 5553, Université de Grenoble, BP 53, 38041 Grenoble cedex 09, France ; [2]Department of Entomology, University of Georgia, Athens, GA 30602-2603, USA ; [3]Vector Group, Liverpool School of Tropical Medicine, Liverpool L3 5QA, UK ; [4]Biochemistry and Molecular Biology, University of Georgia, Athens, GA 30602-2603, USA
Address for correspondence: guillaume.tetreau@gmail.com

The bio-insecticide *Bti* (*Bacillus thuringiensis* var. *israelensis*) is used worldwide against mosquito larvae. The main threat to the long-term use of this safe and efficient bio-insecticide is the potential evolution of resistance in treated mosquito populations. Field-sampled litters containing *Bti* were used for selecting an *Aedes aegypti* laboratory strain. After 20 generations, this strain exhibited only moderate resistance to *Bti*, but consistent resistance (up to 30 fold) to separate *Bti* Cry toxins. Because *Bti* is a mixture of toxins with complex and synergistic modes of action, the resistance is likely to involve several mechanisms, including modifications in genes and/or in their expression. We therefore adopted a combination of transcriptomics and proteomics approaches to elucidate the mechanisms involved. We first performed a whole-transcriptome approach, using next-generation SOLEXA sequencing, to compare the expression level of all the transcripts in resistant versus susceptible whole larvae. Because *Bti* mostly interacts with mosquito larvae in the midgut, we then focused on midgut transcripts using the new Agilent-microarray and on midgut proteins using 2D-DiGE technology. *Bti* resistance appears to involve several categories of genes and proteins, including serine proteases, membrane receptors, but also genes involved in cell signaling processes and immunity. We discuss our results in the light of potential mosquito resistance evolution in the field due to *Bti* environmental persistence and vector control strategies.

Contributed Paper, Monday 15:30 **14**

Fruit and Shoot Borer Resistant Transgenic Bt Brinjal in India

Shekharappa, P.M.Salimath and R.R. Hanchinal
University of Agricultural Sciences, Dharwad-580 005, (Karnataka: India)
Address for correspondence: shekhar1993@yahoo.com

Brinjal or eggplant is very important vegetable in India and known to be attacked by number of insect pests. Among them fruit and shoot borer (FSB) poses serious crop loss (70%). The efforts made by farmers to contain this insect was in vain as it led to environmental pollution also and health hazards due to high pesticide residues to the end users apart from increasing the cost of cultivation. Keeping these things in view Bt brinjal conversion programme was initiated in a public private partnership mode under the aegis of Agriculture Biotechnology Support Project II (ABSPII) of USAID and Department of Biotechnology, Govt. of India with the involvement of M/s. Maharashtra Hybrid Seeds Company (Mahco) (event donor), Tamil Nadu Agricultural University (TNAU), Coimbatore; University of Agricultural Sciences, Dharwad; and Indian Institute of Vegetable Research (IIVR), Varanasi in India. As a part of this multinational consortium University of Philippines, Los Banas (UPLB) and Bangladesh Agricultural Research Institute, Bangladesh, also trying to take the benfit of the technology to needy farmers. Through this royalty free license agreement, the public institutions have converted locally important varieties into transgenic Bt versions and pledge to make them available to resource constrained farmers at actual cost of production. Being varieties, famers will have option to save seeds to subsequent crop. The transgenic Bt brinjal contains *cry 1A*c gene confers resistance against FSB. All Indian partners have tested their products in field for their safety to non-target organisms and agronomy performance besides submitting whole dossier of biosafety as per evolving India Biosafety regulation. Following one year of moratorium on the technology for its field release, GEAC/special panel has resumed discussions while country is all set to have new and more efficient regulatory authority shortly. Bring is shaping India regulatory system and would make way for transgenic food events in days ahead.

Contributed Paper, Monday 15:45 **15**

Field level Resistance in bollworms to Bt cotton in India

Govind T Gujar and Vinay Kalia
Division of Entomology, Indian Agricultural Research Institute, New Delhi 110012
Address for correspondence: gtgujar@yahoo.com

Cotton bollworms have shown the ability to evolve resistance to Bt toxins like Cry1Ac and Cry2Ab. In India, although appearance of bollworms on Bt cotton was reported in press; these reports lacked scientific scrutiny. It was only in 2010 that field level resistance in the pink bollworm, *Pectinophora gossypiella* to Bt cotton expressing Cry1Ac was reported from Gujarat state; as Cry1Ac resistance evolution was indicated in early 2009. Another report in late 2010 from Karnataka mentioned survival of the cotton bollworm, *Helicoverpa armigera* larvae on Bt cotton. These insects completed a full generation on Bt cotton expressing Cry1Ac alone and Bt cotton expressing both toxins viz., Cry1Ac and Cry2Ab. Our studies on resistance monitoring of *H. armigera* showed the ability of progeny of larvae collected from Bt cotton in Anand to be 390-fold resistant vis-à-vis the most susceptible Surat strain and about 43-fold resistant vis-à-vis those from non-Bt cotton in Anand (Gujarat). Further, the outbreaks of *Spodoptera litura* on soybean and Bt cotton were reported in the past; without confirmation of their susceptibility levels to Bt toxins present in Bollgard II cotton. Fortunately, development of Bt resistance in these pests has not led to cotton crop failure. Bt resistance management is now of outmost importance for cotton sustainability in India which grows world's highest area of Bt cotton annually (10.6 million hectares out of total of 11.5 million hectares); produces world's 2nd largest quantity of lint cotton (33 million bales), but ranks lower than world's average in terms of productivity.

Contributed Papers Monday, 14:00-16:00

Virus 1

Contributed Paper, Monday 14:00 **16 STU**

Mutations in 3 amino acids in VP4 affect the virulence of *Junonia coenia densovirus*

Cécilia Multeau[1,2] ; Aurélie. Perrin[1,2] ; Marie E. Pastor[2] ; Rémy Froissart[3] ; Mylène Ogliastro[2]
[1]BIOTOP, Route de Biot - D4, 06560, Valbonne, France, [2]INRA, UMR 1333, Diversity, Genomes and insect-pathogen Interactions, Université Montpellier 2, Place Eugène Bataillon, cc101, 34000, Montpellier, France, [3]CIRAD, UMR-BGPI TA A-54/K, Campus International de Baillarguet, 34398, Montpellier, France
Address for correspondence: cecilia.multeau@supagro.inra.fr

Viruses belonging to the *Densovirus* genus (family *Parvoviridae*) are infectious per orally for Lepidoptera and innocuous for vertebrates, suggesting an interesting potential as biological control agents. To assess for their capacity and their risks of invading new hosts, mechanisms underlying host range and specificity need to be deciphered carefully. Our previous studies described the pathogenesis of the densovirus prototype, *Junonia coenia* DNV on a susceptible host the Lepidoptera *S .frugiperda*. Comparative crystallographic analysis of *JcDNV* and the closely related *GmDNV* revealed that few residues on the capsid might be involved in host specificity (Bruemmer A. et al., 2005). To test this hypothesis, several *JcDNV* carrying mutated residues were constructed and used to infect *S. frugiperda*, to measure the impact on virulence and replication. We characterized capsid mutations conferring a lower virulence, correlated with a lower number of recombinant *JcDNV* genomes quantified at the initiation of replication. Our results suggest a lower efficiency of these mutated viral particles in crossing the midgut barrier, potentially giving new clues to identify the receptors of the midgut cells involved.

Contributed Paper, Monday 14:15 **17 STU**

Molecular characterization of *Autographa californica* multiple nucleopolyhedrovirus ORF43 null mutant

Xue Ying Tao[1], Jae Young Choi[1], Yong Wang[1], Jae Su Kim[2], and Yeon Ho Je[1]
[1] Department of Agricultural Biotechnology, Seoul National University, Seoul 151-742, Korea
[2] Research Institute for Agriculture and Life Sciences, Seoul National University, Seoul 151-742, Korea
Address for correspondence: btrus@snu.ac.kr

ORF43 (*ac43*) of *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) is a highly conserved baculovirus gene whose function is unknown. To determine the role of *ac43* in baculovirus life cycle, we used a new AcMNPV bacmid (bAc-MK) and generated *ac43* deletion virus (*ac43*KO) by using the plasmid capture system (PCS). After transfection into *Spodoptera frugiperda* cells, *ac43*KO produced significantly different OBs which with much larger size; and especially had much single nucleocapsids compared to Ac-MK. Furthermore, *ac43*KO bacmid led to a defect in transcription and expression of *polyhedrin*, which result in less OBs production. However, *ac43*KO didn't affect BV production since there're no remarkable difference of BV titer in both *ac43*KO and Ac-MK. These results demonstrate that *ac43* plays an important role in polyhedrin expression, OB formation, and virion assembly.

Contributed Paper, Monday 14:30 **18 STU**

***Mamestra configurata nucleopolyhedrovirus* (MacoNPV): Potential chitin-binding proteins and their role in oral infectivity**

Amy L. Noakes[1,2]; Cedric Gillott[1]; Dwayne Hegedus[2,3]; David Theilmann[4]; Martin Erlandson[1,2].

[1]Department of Biology, University of Saskatchewan, 112 Science Place, Saskatoon, SK S7N 5E2, Canada; [2]Agriculture and Agri-Food Canada, Saskatoon Research Centre, 107 Science Place, Saskatoon, SK S7N 0X2 Canada; [3]Department of Food and Bioproduct Sciences, University of Saskatchewan, 51 Campus Drive, Saskatoon, SK, S7N 5A2, Canada; [4]Agriculture and Agri-Food Canada, Pacific Agriculture Research Centre, 4200 Highway 97, Summerland, BC V0H 1Z0 Canada.

Address for correspondence: amy.noakes@agr.gc.ca

The host midgut is the preliminary site of baculovirus infections. In order to initiate infection in the midgut epithelium, occluded virions must transit the peritrophic matrix (PM), a protein-chitin meshwork that lines the midgut of most insects. The mechanism by which this happens is unknown. Two MacoNPV proteins, Mc118 and Mc164, have putative chitin-binding domains and may be involved in an interaction between occluded virions and the PM, and therefore may be important for oral infectivity. Chitin-binding activity was assessed for Mc118 and Mc164 using standard chitin bead protocols. Preliminary results indicate that both proteins interact with chitin. To determine the role these proteins play in oral infectivity, gene knockout constructs were created using an established AcMNPV "AcBac" system. The AcMNPV homologues of MacoNPV ORF118 and ORF164, AcMNPV ORF150 and ORF145 respectively, were targeted. The infectivity of the knockout constructs were tested in 4th instar *Trichuplusia ni* larvae using both oral feeding assays and intrahemocelic injections. Initial results indicated that the AcORF145 knockout construct was less infectious by the oral route than the wild type, while the AcORF150 knockout had no effect on oral infectivity. No difference in infectivity compared to wild type was noted when the knockout viruses were injected intrahemocelically. Oral infectivity was partially restored when the AcORF145 knockout was repaired with MacoNPV ORF164. These initial results suggest that AcMNPV ORF145 but not ORF150, is required for normal levels of oral infectivity in *T.ni*.

Contributed Paper, Monday 14:45 **19**

HA44 is an essential gene for *Helicoverpa Armigera* Nucleopolyhedrovirus replication and its coiled-coil domain is functional important

Fei Deng, Sijiani Luo, Yuan Kang, Hualin Wang, Zhihong Hu.

State Key Laboratory of Virology and CAS Key Laboratory of Agricultural and Environmental Microbiology, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan 430071

Address for correspondence: df@wh.iov.cn

ORF44 (*Ha44*)of *Helicoverpa armigera* nucleopolyhedrovirus (HearNPV) has been reported as one of the conserved genes of group II alphabaculovirus and betabaculovirus, encoding a protein located in the nucleocapsid of both BV and ODV. To determine the function of *ha44* in the HearNPV infection, a *ha44*-knockout bacmid and a *ha44*-repaired bacmid were constructed. Transfection and infection analysis demonstrated that *ha44* is an essential gene for HearNPV replication. Quantitative real-time PCR analysis demonstrated that *ha44* deletion did not affect viral DNA synthesis. However, electron microscopy revealed that no nucleocapsid was found in the cells transfected with *ha44*-knockout bacmid, suggesting HA44 plays a role on nucleocapsid assembling. HA44 forms aggregated dots in the infected cells. Sequence analysis revealed a putative coiled-coil domain at 187-255 aa of HA44. A series bacmids containing different mutations at the coiled-coil domain were constructed. The results showed that bacmids with mutation of L183P or L244P were abolished to produce infectious virus. Our study showed that HA44 is an essential gene for HearNPV replication and that the coiled-coil domain is functional important.

Contributed Paper, Monday 15:00 **20**

Baculovirus virion formation requires the interaction between VP80 and the F-actin cytoskeleton to connect the viral replication factory with the nuclear periphery

Martin Marek[2,3], Feana Francis-Devaraj[1], Lionel Galibert[2], Just M. Vlak[1], Otto-Wilhelm Merten[2], Monique M. van Oers[1]

[1] Laboratory of Virology, Wageningen University, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands, [2] Department of Bioprocess Development, Généthon, 1bis, rue de l'Internationale – 91002 Évry Cédex, France, [3] Integrated Structural Biology, IGBMC, 1, rue Laurent Fries, 67404 Illkirch Cedex, France

Address for correspondence: monique.vanoers@wur.nl

Recently we showed that the *Autographa californica* multicapsid nucleopolyhedrovirus VP80 protein is essential for the formation of both budded virus (BV) and occlusion-derived virus (ODV). Nucleocapsid formation is not affected by a *vp80* deletion, but the nucleocapsids no longer migrate from the virogenic stroma to the nuclear periphery. In baculovirus-infected cells, an EGFP-tagged VP80 protein was localized in the nuclei, adjacent to the virus-triggered F-actin scaffold. Interaction between VP80 and actin was confirmed by co-immunoprecipitation. VP80 and actin were present in a highly organized 3D-network physically connecting the virogenic stroma with the nuclear envelope. VP80 is also associated with the nucleocapsid fraction of both BVs and ODVs, typically with one end of the nucleocapsids. The presence of motifs with homology to invertebrate paramyosin proteins further supports the view of a role of VP80 in the transport of nucleocapsids to the nuclear periphery on their way to form BVs and ODVs.

This fundamental information permitted us to engineer a baculovirus expression system that produced foreign proteins at high levels without contaminating baculovirus particles. In the conventional systems baculovirus virions are abundantly present and form a serious hurdle, especially in the manufacture of heterologous virus-like particles or gene delivery vectors, such as AAV. To this aim, a defective baculovirus lacking *vp80* was used and the initial virus inoculum was made in a *trans*-complementing cell line synthesizing the VP80 protein. The *trans*-complemented, defective baculovirus was capable of producing high levels of recombinant protein, whilst the co-production of baculovirus progeny virions was completely inhibited.

Contributed Paper, Monday 15:15 **21**

***Autographa californica nucleopolyhedrovirus ac93* is a previously unidentified core gene which is required for intranuclear microvesicle formation and egress of nucleocapsids from the nucleus**

Meijin Yuan, Zhenqiu Huang, Denghui Wei, Zhaoyang Hu, Kai Yang*, Yi Pang

State Key Laboratory of Biocontrol, Sun Yat-sen University, Guangzhou 510275, China

Address for correspondence: lssymj@mail.sysu.edu.cn

Autographa californica nucleopolyhedrovirus orf93 (*ac93*) is a highly conserved uncharacterized baculovirus gene which has been found in all sequenced baculovirus genomes except *Culex nigripalpus nucleopolyhedrovirus*. In this study, we identified *ac93* as well as another highly conserved gene *ac94* as core genes, thereby presenting *ac92-ac93-ac94* a core gene cluster. To investigate the functional role of *ac93* in baculovirus life cycle, an

ac93-knockout bacmid was constructed via homologous recombination in *Escherichia coli*. Fluorescence and light microscopy showed that the infection of *ac93*-knockout virus could not spread and titration assays confirmed the defect in budded virus production. However, the deletion of *ac93* did not affect viral DNA replication. Electron microscopy indicated that *ac93* is required for egress of nucleocapsids from nucleus and formation of intranuclear microvesicles, which are precursor structures of occlusion-derived virus envelopes. Immunofluorescence analysis showed that Ac93 exhibited a double ring localization pattern with one ring towards the cytoplasmic membrane and another ring in the nuclear ring zone. Our results suggested that *ac93* is a previously unidentified core gene which plays an essential role in the formation of occlusion-derived virus envelope and egress of nucleocapsid from the nucleus.

Contributed Paper, Monday 15:30 **22**

Mutagenesis and functional analysis of N-linked glycosylations of the major envelope fusion protein of *Helicoverpa armigera* Single Nucleocapsid Nucleopolyhedrovirus

Shu Shen[1], Manli Wang[1], Shufen Li[1], Xin Li[1], Fei Deng[1], Just M. Valk[2], Zhihong Hu[1] and Hualin Wang[1*]
State Key Laboratory of Virology and Joint Laboratory of Invertebrate Virology, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan 430071, People's Republic of China [1], Laboratory of Virology, Wageningen University, 6708 PB Wageningen, the Netherlands[2]
Address for correspondence: h.wang@wh.iov.cn

Glycosylation is commonly considered of significant meaning for the functions of glycoprotein during the viral live-cycle. The F protein (HaF) is the principle functional envelope fusion (glyco)protein of *Helicoverpa armigera* Single Nucleocapsid Nucleopolyhedrovirus (HearNPV). Computer-assisted analysis predicted that the HaF is highly N-linked glycosylation protein with five glycosylation sites in F1 fragment and one in F2 fragment. The one N-glycosylation site found in F_2 fragment located at site N104, which was previously characterized being important for viral infectivity and low-pH induced fusogenicity. The functions and the significant of the rest five potential glycosylation sites located in F1 fragment at sites of N293, N361, N526, N571 and N595 are not known. In this study, a sophisticated mutagenesis on these glycosylation sites both located in fragment F1 and F2 are conducted by series mutations at N-glycosylation sites and by construction of recombinant HearNPVs referring to various N-linked glycosylation sites mutated HaFs. Results indicated that most N-glycosylation sites mutated recombinant HaSNPVs (rHaSNPVs) could produce infectious progeny viruses except for that mutant with all glycosylation sites mutated. The N-glycosylations at different sites were found of various affections for the function of F protein and virus properties. The mutations at single site or at multiple-sites (combination) affect the fusogenicity nd/or impaired the proper of the transportation of mutant F proteins in host cells. The mutation at N571 seemly facilitated virus entry into host cells. The mutation as N526 impaired virus production and infectivity as well as fucogenicity and subcellular location of mutated F protein. The mutation at multiple N-glycosylation sites simultaneously was found dramatically interfered the antigenicity of F protein and was found totally impaired the function of mutant F to rescue the GP64-null AcMNPV. In summary, the N-glycosylations in HearNPV F protein was found of significance for the function, fusogenicity and transportation of F protein in host cells and which in consequence affect the virus pathogenesis.

Contributed Paper, Monday 15:30 **23 STU**

***Autographa californica nucleopolyhedrovirus pe38* is required for nucleocapsid production.**

Shiyun Huang, Kai Yang, Yi Pang
State Key Laboratory of Biocontrol, Sun Yat-sen University, Guangzhou 510275, China
Address for correspondence: sharonkam1987@yahoo.com.cn

pe38 gene of *Autographa californica nucleopolyhedrovirus* (AcMNPV) encodes a protein which has been described as a trans-activator in baculovirus. The protein contains a leucine zipper near its carboxyl terminus and a RING finger at its amino terminus. It is reported that *pe38* is important but not necessary for virus production during infection. In this study, we constructed a *pe38* knockout bacmid in which most of the *pe38* open reading frame was replaced with that of the *chloramphenicol acetyltransferase* gene through homologous recombination in *Escherichia coli*. Meanwhile, a *pe38* repair bacmid was constructed by transposing *pe38* open reading frame under the control of its native promoter to the *polyhedrin* locus of the *pe38* knockout bacmid. By transfecting *Spodoptera frugiperda* cells with these viruses, it was indicated that loss of *pe38* led to a defect in production of nucleocapsid, while the *pe38* repair bacmid rescued this defect. Subsequently, different fragments of *pe38* gene, based on the conserved domain analysis, were inserted into the *polyhedrin* locus of the *pe38* knockout bacmid to investigate their role in *pe38*.

Contributed Papers Monday, 14:00-16:00

Microbial Control 1

Contributed Paper, Monday 14:00 **24 STU**

Modulation of immunity in cotton bollworm, *Helicoverpa armigera* (Lepidoptera: Noctuidae)

Bindiya Sachdev[1,2]; Gatikrushna Singh[1]; Neema Agrawal[1]; Rakesh K. Seth[1]; Raj K. Bhatnagar[1]
[1]International Center for Genetic Engineering & Biotechnology (ICGEB), Aruna Asaf Ali Marg, New Delhi-110067, India,
[2]Department of Zoology, University of Delhi, Delhi-110007, India
Address for correspondence: raj@icgeb.res.in

The cotton bollworm, *Helicoverpa armigera*, is a serious global agricultural pest. Its predation on crop plants has been successfully controlled by the application of insecticidal proteins of *Bacillus thuringiensis* (Bt). Perceived threat to emergence of resistance to Bt proteins, necessitates discovery of alternate biomolecules specifically targeting *H. armigera*. Insect larvae are exposed to a variety of microbes during their natural growth and development. Through a robust surveillance system, insect larvae are able to differentiate between the invading microbes and mount an appropriate immune response. The major components of insect challenge include Phenoloxidase cascade and synthesis of anti-microbial peptides. Preliminary data revealed that the extent of innate immune response is different upon exposure to enterobacter, *Photorhabdus luminiscens* or *Escherichia coli*. We have examined the levels of major components of phenoloxidase cascade, prophenoloxidase (PPO) and prophenoloxidase-activating enzyme (PPAE) upon challenge with *P. luminiscens/E. coli.* The extent of upregulation of PPO/PPAE is extremely divergent upon different bacterial challenge. The inter-relationship between PPO-PPAE and coordinated modulation has been examined at various levels of microbial density. The interaction of PPO and PPAE has been investigated by *in vitro* pull down assay and *ex in vivo* yeast two-hybrid assay. By generating a series of deletion mutants of PPO, we have identified the PPAE interacting region of PPO for activation. We have identified that N-terminus propeptide of PPO inhibits cognate enzyme activity. These results will be discussed in the context of immune-compromised pest.

Contributed Paper, Monday 14:15 **25 STU**

Interaction between a beetle and its pathogen: do Asian longhorned beetles behaviorally fever?

Joanna J. Fisher; Ann E. Hajek
Department of Entomology, Cornell University, Ithaca NY 14853-2601
Address for correspondence: jjf236@cornell.edu

Asian longhorned beetles (ALB), *Anoplophora glabripennis* (Motschulsky), from China are invasive woodborers in the Eastern United States with the potential to negatively impact economic and environmental interests in US hardwood forests. ALB have caused extensive tree mortality in China due to the widespread planting of susceptible trees. Entomopathogenic fungi have shown promise in controlling these insects, however as a result of elevating their body temperatures through basking (behavioral fever), insects may be able to fight off or delay infection and this would limit fungal efficacy. The entomopathogenic fungus *Metarhizium brunneum* (= *M. anisopliae*) is under development for control of ALB, but the incidence of behavioral fever in this system has not been investigated. Preliminary data from placing ALB in no-choice and gradient temperature environments shows ALB do not exhibit behavioral fever in response to fungal infection but that higher temperatures may delay infections. The results of this study will provide insight into the feasibility of utilizing *M. brunneum* as a method for controlling and preventing ALB infestations.

Contributed Paper, Monday 14:30 **26**

Behavioural evidence and horizontal transmission of entomopathogenic fungi by infected and non-infected adult emerald ash borer.

George Kyei-Poku; Debbie Gauthier; Carl Nystrom
Great Lakes Forestry Centre, Canadian Forest Service, 1219 Queen Street East, Sault Ste. Marie, Ontario, P6A 2E5, CANADA
Address for correspondence: gkyeipok@NRCan.gc.ca

The emerald ash borer (EAB) *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae) is an invasive beetle species from Asia that has caused extensive mortality of ash trees (Fraxinus spp.) since arriving in North America. Entomopathogenic fungi (EPF) were determined to be the major mortality factor of EAB in Ontario field populations during a survey of EAB pathogens in 2008. Our general aim is to investigate the potential for controlling EAB with these recovered entomopathogenic fungi (EPF) using the autodissemination technique whereby self-contaminated or autoinfected beetles will introduce and spread EPF throughout the beetle populations. It has been ascertained that effective management of any insect pest often benefits from a comprehensive understanding of its normal social behaviours. In addition, efficient mechanism of transmission is a key factor in the ability of entomopathogens to readily pass between conspecifics, and potentially develop epizootics that may lead to a better level of pest suppression. However, there is lack of information on the behaviour of EAB upon coming into contact with pathogen-infected conspecifics and the degree of pathogen-carry-over-effect on conspecifics. We will discuss potential differences in EAB behaviour in the presence of different treatments and to elucidate the mechanism that EAB employs to detect and/or passively transmit fungal pathogens to conspecifics in their habitat.

Contributed Paper, Monday 14:45 **27 STU**

Optimum external sterilization technique using sodium hypochlorite on *Plectris aliena* grubs (Coleoptera: Scarabaeidae) and effects on behavior

Nancy L. Brill, Wes D. Watson, Mark R. Abney
North Carolina State University, Department of Entomology, Raleigh, NC 27695-7630, USA
Address for correspondence: nlbrill@ncsu.edu

Plectris aliena is a white grub that causes severe economic damage to the sweetpotato crop in southeastern North Carolina, US. *Metarhizium anisopliae*, a ubiquitous fungal parasite of many soil dwelling arthropods, has been found to infect *Plectris aliena* and could be an effective fungal biocontrol agent for controlling grub damage. External sterilization of the white grubs is a necessary, initial technique when using field collected grubs for laboratory bioassays that test the pathogenicity of *Metarhizium anisopliae*. External sterilization of *P. aliena* showed that a concentration of 2% sodium hypochlorite and a 2 minute immersion time, or a concentration of 1% sodium hypochlorite and a 3 min immersion time, for the grubs were efficient for preventing bacterial infection. Over a two month period, grubs were more likely to exhibit normal behavior of burying themselves in non-sterile soil (NSS) than sterile soil (SS). Additional treatments of grub immersion in 10% sodium thiosulfate (instead of water) after external sterilization, as well as effects on development in sterile conditions, are also under investigation.

Contributed Paper, Monday 15:00 **28**

The effect of pathogen odor signals on behaviors of termites, *Coptotermes formosanus*

Aya Yanagawa[1]; Nao Fujiwara-Tsujii[2]; Toshiharu Akino[3]; Tsuyoshi Yoshimura[1]; Susumu Shimizu[4]
[1]Research Institute for Sustainable Humanosphere, Kyoto University, Gokashou, Uji, 611-0011, Japan, [2]National Institute of Agrobiological Science, Ohwashi, Tsukuba, 305-0851, Japan, [3]Department of Biology, Kyoto Institute of Technology, Matsugasaki, Kyoto, 606-8585, Japan, [4] Institute of Biological Control, Faculty of Agriculture, Kyushu University, Fukuoka, 812-8581, Japan
Address for correspondence: ayanagawa@rish.kyoto-u.ac.jp

Entomopathogen-resistant behaviors of termites are known as commonly as other social insects, and often account for the difficulty in controlling termites by fungi. We studied the behavioral response of *Coptotermes formosanus* to six isolates of entomopathogenic fungus, *Metarhizium anisopliae* 455, *M. anisopliae* UZ, *Beauveria brongniartii* 782, *B. bassiana* F1214, *Paecilomyces fumosoroseus* K3 *and P. fumosoroseus* 8555 with different levels of virulence for better understanding of the behavioral reaction of termites to fungi in the nature. The reactions of termite against pathogenic fungal odor were examined for short and long terms. For the short term tests, Y tube test was conducted to know their most quick reaction to the fungal odor. In addition one fungal- or odor-treated termite was mixed with its four nestmates and the frequency of grooming behavior in the group was observed for 15 minutes. As for long term tests, the behaviors of termite groups which has one fungal- or odor-treated nestmate were observed for a week to compared their attack, cannibalism and burial behaviors between the groups. The results suggest that termite avoid the fungal odor at the most early 'encountered stage', and the odor information enhanced the grooming and attack behaviors. The behavioral changes seem to be related with odor information. Virulence did not induce any special virulence-dependent behavior.

Contributed Paper, Monday 15:15 **29**

A push-pull biocontrol strategy involving Chinese cabbage, red clover *Entomophthora muscae* and the cabbage- and turnip root fly

Ingeborg Klingen, Maria Björkman, Pierre Antoine Allard, Idun Bratberg, Richard Meadow
Norwegian Institute for Agricultural and Environmental Research (Bioforsk), Plant Health and Plant Protection Division, Høgskoleveien 7, N-1432 Ås, Norway.
Address for correspondence: ingeborg.klingen@bioforsk.no

The use of Chinese cabbage as a trap crop where insect pathogenic fungi may prolifereate has been tested in a series of push-pull strategy experiments both in the laboratory and in the field. The pest species studied are the cabbage- and turnip root fly (*Delia radicum* and *D. floralis*). In a dual choice laboratory experiment, both healthy and *Entomophthora muscae* inoculated *D. floralis* were tested for choice of plant for oviposition. The choices were 1) Broccoli against Broccoli 2) Chinese cabbage against Broccoli 3) Broccoli against Broccoli under sown with clover 4) Chinese cabbage against Broccoli under sown with clover. In a semi-field pilot study with Broccoli and Chinese cabbage the choice between main - and trap crop for healthy and inoculated flies, as well as fungal transmission between flies over time, was studied. A pilot field study has also been performed to investigate the overall effect of using Chinese cabbage as a trap crop as well as studying the spatial distribution of *Delia* eggs in a cabbage field. The results from the dual choice experiment and both pilot studies indicates that using Chinese cabbage as a trap crop is a promising strategy for the management of *D. radicum* and *D. floralis*, both as a oviposition attractant and as a trap crop where insect pathogenic fungi may proliferate and kill the adult flies.

Contributed Paper, Monday 15:30 **30**

Potential synergism between *Beauveria bassiana* and plant extract of *Ginkgo biloba* against the two-spotted spider mite, *Tetranychus urticae* (Acari: Tetranychidae)

Farzaneh Seyed-Talebi[1]; Reza Talaei-Hassanloui[2]; Katayoon Kheradmand[1]
[1]Department of Plant Protection, College of Abureyhan, University of Tehran, Pakdasht, Iran; [2] Department of Plant Protection, College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran.
Address for correspondence: rtalaei@ut.ac.ir

The two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae), is a serious cosmopolitan pest species commonly found on many agricultural crops. Biological control agents including *Beauveria bassiana* (Bals.) Vuill. are expected to reduce the dependence on synthetic acaricides, either as a stand-alone solution or as a component of an IPM system for pest control. Our preliminary tests indicated that seed extract of a medicinal plant, *Ginkgo biloba* L. (Ginkgoaceae) has acaricidal properties on *T. urticae* and has no deleterious effect at low concentrations on conidial germination and mycelial growth of a native strain of *B. bassiana*. In this research, the potential synergism between *B. bassiana* EUT105 and ether extract of *G. biloba* seed's core was studied for the acaricidal activity on *T. urticae* adult females. Three concentrations (5, 10 and 20%) of plant extract and LC_{50} value of *B. bassiana* (10^7 conidia/ml) were considered in assays. The mites were sprayed with 1.5 ml of each treat concentration using a Potter tower. The experiment was repeated twice to ensure reproducibility of the results. There were significant differences among treatments for mortality causes on *T. urticae* adults. Mortality was increased when *B. bassiana* sprayed in combination with plant extract. Mean percentages were 12.8, 23.7, 50.9, 87.3 and 94.5% for *G. biloba* 5%, *G. biloba* 10%, *B .bassiana* alone, *G. biloba* 5 % + *B.bassiana* and *G. biloba* 10 % + *B.bassiana*, respectively. Although there are very few reports for fungicidal effect of *G. biloba* compounds on some plant pathogen but our results indicate that this plant extract has a synergist effect on *B. bassiana* for control of *T. urticae* while no inhibitory effect on conidial germination and mycelia growth at normal levels. Possible reasons to the results are discussed.

Contributed Paper, Monday 15:45 **31**

Fungus in an oily residue – the perfect solution for a healthy home…

Nina E. Jenkins[1]; Simon Blanford[1,2]; Maureen Coetzee[3]; Brian Chan[2]; Andrew Read[2] & Matthew B. Thomas[1]
[1]Department of Entomology, Merkle Lab, The Pennsylvania State University, State College, PA 16802, USA, [2]Department of Biology, The Pennsylvania State University, State College, PA 16802, USA, [3]Malaria Entomology Research Unit, University of the Witwatersrand and the National, Institute for Communicable Diseases, Private Bag X4, 1 Modderfontein Road, Sandringham 2131, South Africa.
Address for correspondence: nej2@psu.edu

Mosquitoes are susceptible to infection by entomopathogenic fungi such as *Beauveria bassiana* and *Metarhizium* spp. following contact with sprayed surfaces, and have been shown to be less able to transmit malaria as a result of fungal infection (Blanford *et al*, 2005). Conidia of entomopathogenic fungi are easily formulated in mineral oil, and can be applied to a range of surfaces around the home where mosquitoes commonly alight. However, local ambient temperature and humidity conditions in malaria-affected countries can be unfavourable for conidial survival even inside houses/huts. Furthermore, different surfaces and building materials may also have an impact on conidial survival. Here we present the results of a series of assays conducted at high (80%) humidity and temperatures (22, 26, 32 deg C). Oil formulated *B. bassiana* conidia were applied to a range of surfaces (wood, clay/mud, cement) and efficacy of the spray was measured over time by exposing mosquitoes periodically to these surfaces and monitoring for mortality due to fungal infection. Alongside these studies, we monitored survival of unformulated conidia under the same humidity and temperature range using direct germination count to determine viability over time. Our results demonstrate that *B. bassiana* offers great potential for use as a residual spray for malaria control.

Contributed Papers Monday, 16:30-18:30

Viruses 2

Contributed Paper, Monday 16:30 **32**

Deciphering the genetic and biological plasticity of *Cydia pomonella* granulovirus (CPGV)

Karolin E. Eberle[1], Stefanie Schulze-Bopp[2], Eva Fritsch[1], Karin Undorf-Spahn[1], Jutta Kienzle[3,] Johannes A. Jehle[1,2]
[1]Julius Kühn-Institut, Bundesforschungsinstitut für Kulturpflanzen, Institut für Biologischen Pflanzenschutz, Heinrichstr. 243, 64287 Darmstadt, Germany; [2]1Laboratory of Biotechnical Crop Protection, Department of Phytopathology, Agricultural Service Center Palatinate (DLR Rheinpfalz), Breitenweg 71, 67435 Neustadt/Weinstrasse, Germany; [3]Fördergemeinschaft Ökologischer Obstbau e.V.
Address for correspondence:.jehle@jki.bund.de

The *Cydia pomonella* granulovirus (CpGV) is one of the most important biological control agents for codling moth. We performed genome sequencing and phylogenetic analyses of several different CpGV isolates, such as CpGV-M, -I12, -E2, -S, -I07. These analyses showed that the isolates belong to different genome types and differ in single nucleotiude polymorphisms (SNPs) and indel mutation of coding and non-coding genomic regions.

When resistance to CpGV was observed in European orchards, it could be attributed to a single resistance allele which is dominantly inherited on sex-chromosome Z. Several CpGV isolates (CpGV-I12, -S, -E2, MadexPlus) overcome this resistance. From whole genome sequencing, it could be concluded that the observed CpGV resistance is not a resistance in general, but to type A genomes like CpGV-M. Interestingly, a further type of resistance which cannot be overcome by Madex Plus, CpGV-I12 or -S was observed in two German orchards. Crossing experiments between resistant individuals and a susceptible laboratory colony showed that the inheritance of this resistance did not follow the previously described pattern of Z-linked, dominant resistance. The offspring of some crossings was completely resistant to CpGV-M and -S, but in some individuals, CpGV-M caused even higher mortality than the resistance overcoming CpGV-S. On the other hand, another CpGV isolate CpGV-V15 caused high mortality in these populations in both bioassays and in the field. We aim to elucidate this complexity in the interaction of various CpGV and different codling moth population on molecular basis. The molecular mechanisms of interaction of different CpGV isolates in codling moth is of enormous interest in terms of CpGV evolution, and it is of crucial importance for the application of CpGV in the field.

Contributed Paper, Monday 16:45 **33 STU**

The *Agrotis* baculovirus complex: multiple viruses for multiple pests

Jörg T. Wennmann[1]; Wael El-Menofy[2]; Waly Essam[2]; Naglaa Abdallah[2]; Johannes A. Jehle[1]

[1] Institute for Biological Control, Julius Kühn-Institute, Federal Research Centre for Cultivated Plants, Darmstadt, Germany, [2] Faculty of Agriculture, Cairo University, Giza, Egypt

Address for correspondence: joerg.wennmann@jki.bund.de

Larvae of the genus *Agrotis* (Lepidoptera: Noctuidae) are known to be severe soil pests on a wide range of field crops and vegetables in Europe, Asia and Africa. *Agrotis spec.* are highly susceptible for a broad number of baculoviruses and in the past, two Alphabaculoviruses (AgseNPV-A (Polish strain), AgseNPV-B (Oxford strain)) and one Betabaculovirus (AgseGV) were isolated from the common cutworm *A. segetu*m. From larvae of the black cutworm *A. ipsilon* another Alphabaculovirus, *Agrotis ipsilon* nucleopolyhedrovirus (AgipNPV, Illinois strain), was isolated. Bioassay analysis demonstrated the cross-infectivity of all four baculoviruses for both hosts, which made them potential biocontrol agents for the control of cutworms. Especially in terms of resistance management the usage of a combination of different baculoviruses is regarded to be useful. In order to develop methods for identification of the different viruses we developed a multiplex polymerase chain reaction (PCR) and quantitative PCR (qPCR) based method. The genome of AgseNPV-B was completely sequenced and a comparative genome analysis of AgseNPV-B, AgseNPV-A and AgipNPV was conducted. Phylogenetic analysis confirmed the close relationship of AgseNPV-B and AgipNPV by a high sequence similarity, although the genome length and number of open reading frames (ORF) of AgseNPV-B and AgseNPV-A were more alike. For biological characterization bioassays and the determination of the median lethal dose (LC_{50}) of AgipNPV and AgseNPV for their common host *A. segetum*, were performed. This work is the basis to analyze the molecular and cellular interaction of these viruses in mixed infections and to optimize the application of these viruses for *Agrotis* control.

Contributed Paper, Monday 17:00 **34**

The ecology of *Spodoptera exempta* nucleopolyhedrovirus within field populations of a migratory pest

Robert I. Graham[1]; David Grzywacz[2]; Wilfred L. Mushobozi[3]; Jennifer S. Cory[4]; Alan D. Shirras[1]; Yamini Tummala[1]; Kenneth Wilson[1]

[1]Lancaster Environment Centre, Lancaster University, Lancaster, LA1 4YQ, UK; [2]Natural Resources Institute, University of Greenwich at Medway, Central Avenue, Chatham Maritime, Kent, ME4 4TB, UK; [3]EcoAgriConsultancy Services Ltd, Nairobi Road, Kwa Idd, PO Box 15040 Arusha, Tanzania; [4]Department of Biological Sciences, Simon Fraser University, Burnaby, BC, V5A 1S6, Canada

Address for correspondence: r.graham@lancaster.ac.uk

The African armyworm *Spodoptera exempta* (Lepidoptera: Noctuidae) is one of the most devastating agricultural pests in sub-Saharan Africa, destroying staple crops such as maize, wheat, sorghum, millet, rice and pasture grasses. This study focuses on the ecology of a natural disease, *S. exempta* nucleopolyhedrovirus (SpexNPV), and examining how this virus may ultimately be utilised within a strategic control program. Over 50 outbreaking larval populations of armyworm were sampled in Tanzania over a four-year period. In addition, a pheromone-trap network was established throughout the country to catch samples of male adults. Natural epizootics of SpexNPV were found to be prevalent in larval outbreaks, causing up to 17% mortality. The genetic structure of these SpexNPV populations was found to be highly variable, both temporally and spatially, with 69 genetic variants of SpexNPV isolated. Laboratory bioassays were undertaken to examine phenotypic variability between these genetic variants. In addition to overt disease, field populations of armyworm harbour a covert persistent infection of SpexNPV that is transmitted vertically from parent to offspring. A quantitative real-time PCR protocol was developed to examine the prevalence of covert viruses within both adult and larval populations, and also to establish viral-load within individual field-collected insects. Our results indicate an intimate host-pathogen relationship within this species, which may be utilised in future strategic biological control and integrated pest management programs.

Contributed Paper, Monday 17:15 **35**

SeMNPV reactivation through stress factors in covertly infected *Spodoptera exigua*

C. Virto [1], R. Murillo[1,2], T.Williams[3], P. Caballero[1,2]

[1] Instituto de Agrobiotecnología, CSIC-Gobierno de Navarra, Ctra. de Mutilva s/n 31192, Mutilva baja, Spain; [2] Departamento de Producción Agraria, Universidad Pública de Navarra, Pamplona 31006, Spain; [3] Instituto de Ecología AC, Xalapa 91070, Ver., Mexico

Address for correspondence: pcm92@unavarra.es

Spontaneous reactivation of baculovirus has been postulated as the main cause to explain outbreaks in laboratory insect cultures that have never been exposed to virus and in the development of epizootics in natural populations. Recent studies have reported a high prevalence of baculovirus covert infections in lepidopteran populations, such as *Spodoptera exigua*. Hence, reactivation may be considered as a potential strategy in biological control programs provided the factors involved in triggering patent infections can be elucidated. The aim of this study is to evaluate the effect of different stress factors on covertly infected *S. exigua* larvae in terms of virus reactivation. For this, adult survivors that had ingested occlusion bodies of *S. exigua* multiple nucleopolyhedrovirus (SeMNPV) were mated and the subsequent generation (F1) tested for virus reactivation in the second instar. A number of treatments were tested: i) chemical stressors: 1-01.% copper sulfate, 1% iron sulfate, 1-0.1% hydroxylamine, 2% Tinopal, 1 ppm paraquat dichloride, 1 ppm sodium selenite; ii) inoculation with: SeMNPV-US2, *Chryxodeisis chalcites* NPV (non permissive) and *Bacillus thuringiensis* (spores and crystals); and iii) rearing temperatures of 18 ºC and 28ºC. Both, parental and F1 adults were confirmed to harbor the infection by qPCR. Reactivation was observed in 0.1% copper sulfate, 1% iron sulfate, and 1 ppm selenium treatment that resulted in 12, 15, and 41%

mortality due to SeMNPV, while no larvae with symptoms of viral infection were registered in virus-free controls. Experiments are now being performed to assess reactivation by these substances on descendents of adults that acquired the infections under semi-field conditions.

Contributed Paper, Monday 17:30 **36**

Virus persistence and the dynamics of covert infection in cyclic populations of tent caterpillars

Jenny S. Cory[1]; Jutta Buchhop[1]; Judith H. Myers[2]
[1]Department of Biological Sciences, Simon Fraser University, 8888 University Drive, Burnaby, BC, V5A 1S6 Canada,
[2]Department of Zoology, University of British Columbia, BC, V6T 1Z4 Canada.
Address for correspondence: jsc21@sfu.ca

Epizootics of baculovirus in outbreak populations of Western tent caterpillars, *Malacosoma californicum pluviale* (Dyar) represent a classic example of the importance of disease to cyclic host population dynamics of a forest lepidopteran. A remaining mystery for understanding these dynamics is how disease persists in the system when host populations are low. Nucleopolyhedroviruses (NPVs) are assumed to persist via environmental contamination, but the extremely low densities of apparently disease-free tent caterpillars during the population trough make this mechanism doubtful. An alternative route for NPV persistence is through vertical transmission from parent to offspring. Recently, several studies of other Lepidoptera have shown that covert infection of adults can be common in field populations. Here we examine the evidence for vertically transmitted covert infection in the western tent caterpillars. Annual collections of larvae from field population sites in British Columbia have been reared in the laboratory to assess the level of overt viral infection in the populations. Surviving adults were frozen and have been examined for the presence of virus using PCR, RT-PCR and real time PCR, targeting three genes. Virus appears to persist in a portion of moths and the prevalence and level of persistent virus follows the host population density trajectory.

Contributed Paper, Monday 17:45 **37 STU**

Horizontal gene transfers between baculoviruses and entomopoxviruses

Julien Thézé[1]; Julie Gallais[1]; Jun Takatsuka[2]; Madoka Nakai[3]; Elisabeth Herniou[1]
[1]Insect Biology Research Institute, UMR CNRS-6035, University François Rabelais, 37200 Tours, France, [2]Forestry and Forest Products Research Institute, Matsunosato 1, Tsukuba 305-8687, Japan, [3]Institute of Agriculture, Tokyo University of Agriculture and Technology, Saiwai, Fuchu, Tokyo, 183-8509, Japan.

Adoxophyes honmai (Lepidoptera: Tortricidae) is one of the most important pests of tea plants in Japan. Numerous studies have been led to establish biocontrol applications. An entomopoxvirus (AdhoEPV) and two baculoviruses (AdhoNPV and AdhoGV) have been isolated from diseased *A. honmai* larvae. AdhoEPV has a high prevalence to *A. honmai* larvae. Mixed infections with AdhoNPV or/and AdhoGV are thus possible in the field. Since these viruses infect simultaneously the same host species, do they share genomic homologies for host adaptation? To answer this question, the genome of AdhoEPV was sequenced and annotated. We identified 8 genes showing homologies with baculoviruses. Notably, we found two inhibitor of apoptosis genes (iap) with high similarities to baculovirus homologues, which are known to play an important role in baculovirus response to host immune system. We performed phylogenetic analyses on the identified iap genes, including homologues in entomopoxviruses, baculoviruses and cellular organisms. The highly supported phylogenies showed that the closest relatives to AdhoEPV iap genes are those of AdhoNPV and AdhoGV. Previous studies have shown that baculoviruses had captured iap genes from insects, improving host adaptation. Here, we propose that horizontal gene transfers are possible between evolutionary distant viruses, broadening viral strategies in host adaptation.

Contributed Paper, Monday 18:00 **38**

Genome sequence of a nudivirus from the crane fly *Tipula oleracea*

Annie Bézier[1]; Julien Thézé[1]; Elisabeth Herniou[1]
[1]Insect Biology Research Institute, UMR CNRS-6035, University François Rabelais, 37200 Tours, France.
Address for correspondence: elisabeth.herniou@uni-tours.fr

We sequenced the genome of a dsDNA virus isolated from the crane fly *Tipula oleracea* (Diptera, Tipulidae) by KM Smith in the 1950s. Nucleopolyhedrosis has been described from the closely related species *T. paludosa.* The current taxonomy of the family *Baculoviridae* comprising a Diptera specific genus, the *Gammabaculovirus,* we postulated that this virus could be related to the mosquito virus CuniNPV. This could have shed new lights on specific gene content in the genus. After whole genome amplification we obtained 454 sequences for the genome. The assembly produced a 146 kb contig, with a GC content of 37%. Genome annotation predicted 126 protein-coding open reading frames (ORFs). On one hand, similarity searches revealed that only 19 ORFs are similar to baculovirus core genes. These correspond to the set of core genes shared by baculoviruses and nudiviruses. On the other hand, 11 genes only have homologues in nudiviruses. Lastly, phylogenomic analyses of the core genes clearly showed that this crane fly virus belongs to the nudivirus clade. Furthermore *Tipula oleracea* nudivirus (ToNV) is more closely related to the nudivirus of Helicoverpa zea than to those of *Oryctes rhinoceros* or *Gryllus bimaculatus*.

Contributed Papers Monday, 16:30-18:30

Fungi 1

Contributed Paper, Monday 16:30 **39**

Genetic diversity of *Beauveria* isolates collected from infected pollen beetles (*Meligethes aeneus)*

Nicolai V. Meyling[1], Christina Pilz[2], Siegfried Keller[2], Franco Widmer[3] and Jürg Enkerli[3]
[1] Department of Agriculture and Ecology, University of Copenhagen, Denmark.
[2], Ecological Plant Protection, Agroscope Reckenholz-Tänikon, Reckenholzstrasse 191, Zurich, Switzerland.
[3] Molecular Ecology, Agroscope Reckenholz-Tänikon, Reckenholzstrasse 191, Zurich, Switzerland.
Address for correspondence: nvm@life.ku.dk

Pollen beetles *Meligethes aeneus* F. (Coleoptera, Nitidulidae) migrate from their overwintering sites in hedgerows and forested areas to oilseed rape fields. In the oilseed rape, they feed on flowers and oviposit into flower buds thereby severely reducing seed production. A survey on entomopathogenic fungal infections performed at different locations in Switzerland revealed that *M. aeneus* was exclusively infected by *Beauveria* spp. The goal of the present study was to genetically type 32 isolates of *Beauveria* spp. collected during this survey based on the current molecular phylogeny of *Beauveria* and to assess genetic diversity in the collection. Investigations were based on sequence analyses of the internal transcribed spacer region of the rRNA gene cluster (ITS), the 5'- intron rich region of elongation factor 1-alpha (EF1-α) and the bloc region as well as analysis of 15 simple sequence repeat (SSR) markers. Results revealed that 10 isolates belonged to *B. bassiana* clade Eu_1, three to *B. bassiana* clade Eu_4, 18 isolates belonged to *Beauveria* Clade C, and one isolate was identified as *B. brongniartii*. Amplification success for the SSR markers as well

as allele sizes and locus specific variability varied among the different clades and only four of the 15 markers could be amplified from all isolates. Bioassays performed with 14 isolates representing the different clades revealed a strong variation in virulence among and within the different clades. Results of this study showed that fungal infections of pollen beetles in Switzerland can be caused by genetically diverse *Beauveria* isolates, a fungal genus already established in biological control of various pest insects.

Contributed Paper, Monday 16:45 **40 STU**

House flies (*Musca domestica L.)* delay fungal pathogenesis by fevering – at a cost

Robert D. Anderson[1]; Simon Blanford[1,2]; Matthew B. Thomas[1,2]
[1]Department of Entomology, 501 Agriculture Sciences and Industries Building, Penn State University, University Park, PA 16802
[2]Center for Infectious Disease Dynamics, Penn State University, University Park, PA 16802
Address for correspondence: rda138@psu.edu

The progression of disease in ectotherms is strongly influenced by environmental temperature, however, exactly what temperature an infected host experiences depends on specific aspects of the local microclimate, as well as host behavior. Many ectotherms are able to invoke a "behavioral fever" when infected with a pathogen; by exploiting warmer microclimates, individuals can effectively raise their body temperature above their normal thermal optimum. Although not an uncommon phenomenon, the relative costs and benefits of behavioral fevers have rarely been quantified. Here, we report on a study investigating the nature and consequences of behavioral fever in the house fly, *Musca domestica*, in response to infection with the fungal entomopathogen, *Beauveria bassiana*. We found that infected flies preferred higher temperatures and allocated more time to thermoregulation than uninfected flies. This altered thermal behavior allowed infected flies to significantly extend their survival, which allowed females to lay more eggs relative to infected flies maintained under constant temperatures. However, fevering also imposed costs in terms of lowered egg viability and increased metabolic rate. These results highlight the importance of understanding the interaction between the biology and behavior of the host, pathogen and the environment in the ecology and evolution of ectotherm host-pathogen interactions.

Contributed Paper, Monday 17:00 **41 STU**

Evidence for self-medication: host plant choice of an oligolectic bee and pathogenic *Ascosphaera* spp.

Anja Amtoft Wynns, Annette Bruun Jensen, Jørgen Eilenberg.
Center for Social Evolution, Department of Agriculture and Ecology, University of Copenhagen, Thorvaldsensvej 40, 1871 Frederiksberg C, Denmark.
Address for correspondence: aaw@life.ku.dk

Solitary bees, along with their social relatives the honey bees, suffer from chalkbrood, a larval disease caused by several species of *Ascosphaera* (Ascomycota, Onygenales). Chalkbrood is common in the nests of the solitary bees *Osmia rufa* and *Megachile rotundata* but has never been observed in the related species *Chelostoma florisomne*. *Chelostoma florisomne* is a solitary bee that nests gregariously in the reeds of thatched roofs in Europe: it is oligolectic, provisioning its eggs almost exclusively with *Ranunculus* pollen. *Ranunculus* contains protoanemonin, a compound that exhibits both antifungal and antibacterial activity. We suspected protoanemonin might play role in the apparent absence of chalkbrood in *C. florisomne* and carried out several experiments to test our hypothesis. We switched the provisions of *C. florisomne* and *O. rufa* and found that a diet of *Ranunculus* pollen substantially reduces the incidence of chalkbrood in both taxa. Ethanolic extracts of *Ranunculus* pollen significantly inhibit *in vitro* spore germination of pathogenic *Ascosphaerae*. The evolution of a narrow host plant diet in bees is thought to be linked to nutrition and the recognition of floral morphology. Our results provide evidence that host plant choice in oligolectic bees may also be partly driven by a mean of self-medication.

Contributed Paper, Monday 17:15 **42**

Secretome of Entomophthorales infected aphids documents high pathogen activity and weak host response

Annette B. Jensen[1]; Morten N. Grell[2]; Peter B. Olsen[3]; Lene Lange[2]; Jørgen Eilenberg[1]
[1]Department of Agriculture and Ecology, University of Copenhagen, Thorvaldsensvej 40, DK-1871 Frederiksberg C, Denmark; [2]Section for Sustainable Biotechnology, Aalborg University, Lautrupvang 15, DK-2750 Ballerup, Denmark, [3]Molecular Biotechnology, Novozymes A/S, Krogshøjvej 36, DK-2880 Bagsværd, Denmark
Address for correspondence: abj@life.ku.dk

The fight between host and pathogen at the cellular level is mediated by secreted or membrane bound molecules. Analysing the secretome, therefore, contributes to the understanding of host-pathogen interactions. We found a rich diversity of secreted proteins from the interaction between the grain aphid *Sitobion avenae* and entomophthoralean fungi (*Entomophthora planchoniana*, *Pandora neoaphidis* and *Conidiobolus obscurus*). An advanced method, transposon assisted signal trapping TAST, was used to screen a cDNA library. This screening method has two major advantages; 1) it enriches the library with cDNAs encoding secreted proteins by selecting clones encoding full length proteins with a N-terminal signal peptide and 2) it is unbiased of known sequences and/or functions. A cDNA library was constructed directly from field sampled aphids, which were collected immediately after the first external signs of infection were visible. We show for the first time that fungi from the genera *Pandora* and *Entomophthora* are armed with a battery of hydrolytic enzymes for penetration of the host cuticle, enabling both accesses to the hemolymph and exit for sporulation. Further, the entomophthoralean fungi secrete enzymes, most notably a number of lipases, for digestion of easily accessible high energy compounds within the hemolymph. Interestingly, we identified only few host genes inferred to be involved in the interaction, indicating that aphids only respond weakly to the presence of the pathogens and supporting recent findings that aphids have a reduced immune repertoire.

Contributed Paper, Monday 17:30 **43**

Target-oriented dissemination of *Beauveria bassiana* conidia using predators

Hong Zhu[1], Jeong Jun Kim[1], Hong-Hyun Park[2] and Sang-Guei Lee[2]
[1] Agricultural Microbiology Team, NAAS, RDA, Korea, [2] Division of Crop Protection, NAAS, RDA, Korea
Addresses for correspondence: jjkim66@korea.kr; jjkim66@hotmail.com

Microbial pest control agents are generally applied with the same type of sprayer as chemical pesticides. Such sprayers can negatively influence spore viability because of high temperatures when conidia pass through the nozzle. The dissemination of microbial control agents via predators may have advantages for safe spore dispersal to targeted pests with the added benefit of predation. We conducted a laboratory study to test the target-oriented dispersal of conidia of *Beauveria bassiana* using larvae of the Asian lady beetle and lacewings to control aphids. The predators were inoculated by releasing into Petri dishes containing dry conidia or aqueous spore suspensions.

It was found that the predators dispersed dry conidia more readily than aqueous spore suspensions. Maximum number of conidia attached in about 7 min when the predator larvae were exposed to dried conidia. After release the treated predators on leaves of Chinese cabbage, lacewing larvae dispersed 89% of the conidia attached to their bodies while Asian lady bird beetles dispersed 93%within 12 hours. Both predators dispersed spores up to 2.4 m from the release site. Leaf disc bioassays were conducted to compare two spray methods; the dissemination of conidia of *B. bassiana* by predators and the direct spraying of conidial suspensions. Mortality in sprayed aphids was 91±2.1%. When spores were disseminated by lacewing and lady bird beetles, aphid mortalities were 88±2.1% and 84±4.2%, respectively. Predation was not affected in treated lacewing larvae whereas there was a 20% reduction in predation by lady beetle larvae. It appears that *B. bassiana* can be effectively delivered using certain insect predators.

Contributed Paper, Monday 17:45 **44**

A novel nuptially-transmitted fungal symbiont of *Tenebrio molitor*

Jeffrey C. Lord
USDA, Agricultural Research Service, Center for Grain and Animal Health Research, 1515 College Avenue, Manhattan KS 66502, USA
Address for correspondence: jeff.lord@ars.usda.gov

The yellow mealworm, *Tenebrio molitor*, harbors a symbiont that has spores with a thick, laminated wall and infects the fat body and ventral nerve chord of adult and larval beetles. In adult males, there is heavy infection of the epithelial cells of the testes and between testes lobes with occasional penetration of the lobes. Spores are enveloped in the spermatophores when they are formed at the time of mating and transferred to the female's bursa copulatrix. Infection has not been found in the ovaries. This suggests that there is venereal transmission and per os transmission via externally contaminated eggs. There is little or no pathogenicity. Ribosomal DNA sequencing shows affinity with Basidiomycota.

Poster Papers Monday, 16:30-18:30

Bacteria

Poster / Bacteria, Monday 16:00 **B1 STU**

Construction of cDNA library and cloning of V-ATPase subunit B in midgut from *Helicoverpa armigera* (Hübner)

Langyun Zou, Yanhui Lu, Yan Zhang, Gemei Liang, Kongming Wu, Yuyuan Guo
State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193, P.R. China
Address for correspondence: gmliang@ippcaas.cn

The larval midgut cDNA library of cotton bollworm *Helicoverpa armigera* (Hübner) were constructed using the Switching Mechanism at 5′-end of the RNA Transcript (SMART) technique. The quality evaluation showed the library had a complexity of 2×10^6 pfu/mL, and the recombination rate was 100%. The average length of inserted cDNA fragments was over 1000 bp and 50% were full-length form. About 1098 expressed sequence tags (ESTs) were generated successfully after sequencing. These suggested that one high quality cDNA library of the larval midgut of *H. armigera* had been constructed. After screening the cDNA library, many taqs of Vacuolar ATP enzyme were found. Vacuolar ATP enzyme is localized on the apical membrane of goblet cells and represents the primary energy source for secretion and absorption by serving as an H+/K+ electrogenic transporter across the insect midgut epithelium. A subunit of the midgut V-ATPase has been identified as an intracellular Cry1Ac-binding protein before. In the present work, according the sequences from cDNA library results, the primers of V-ATPase subunit B gene were designed, and full length of V-ATPase subunit B was cloned by degenerated PCR combined with RACE technique (GenBank accession no. GU370066). After expressed in prokaryotic cell, Ligand blot and Western blot analysis revealed that V-ATPase subunit B could bind with *Bacillus thuringiensis* Cry1Ac, Cry2Ab, Cry1C, but not bind to Cry1B.

Poster / Bacteria, Monday 16:00 **B2**

Activation process of Coleopteracidal Cry8 on alder leaf beetle

Shin-ichiro Asano, Keika Yamada, Takuya Yamaguchi, Ken Sahara and Hisanori Bando
Division of Agrobiology, Graduate School of Agriculture, Hokkaido University, N9W9 Sapporo Hokkaido, 060-8589, Japan)
Address for correspondence: sangaku@abs.agr.hokudai.ac.jp

Bacillus thuringiensis (Bt) is a rod shaped, gram positive, spore-forming bacterium. Bt produces parasporal crystal proteins during sporulation. Bt is widely used as pest control agents, the crystal proteins often show insecticidal activity to various harmful pests of Lepidoptera, Diptera and Coleoptera orders. Leaf beetles are important Coleoptera pests because their leaf feeding causes serious damage to forest and crops. Cry8D have high insecticidal activity to some adult leaf beetle species such as alder leaf beetle, rice leaf beetle, and Japanese aspen leaf beetle. We focused on processing patterns by midgut juice. Activation process of alder leaf beetle between Cry8Da and Cry8Ca were compared. Cry8Da was activated and became 64kDa toxin, on the other hands, Cry8Ca was excessive degraded. With heterologous competition assay which used core 64kDa toxins of Cry8Ca and Cry8Da, it found that two toxins recognized a similar receptor in alder leaf beetle's BBMVs. These results reveal that the activation process of Cry8 is more important than Cry8 receptor binding is essential for toxicity.

Poster / Bacteria, Monday 16:00 **B3 STU**

Characterization of field-evolved resistance to transgenic Bt corn in *Spodoptera frugiperda*

Siva R. K. Jakka[1], Cris Oppert[1], Carlos Blanco[2], Maribel Portilla[3], and Juan L. Jurat-Fuentes[1]
[1]Department of Entomology and Plant Pathology, University of Tennessee, Knoxville, TN 37996, USA; [2]USDA-APHIS, Biotechnology Regulatory Services, Riverdale, MD 20787, USA; [3]USDA-ARS, Southern Insect Management Research Unit, Stoneville, MS 38776, USA
Address for correspondence: jurat@utk.edu

Transgenic crops producing toxins from *Bacillus thuringiensis* (*Bt*) have been successfully used for efficient and environmentally safe insect pest control for more than a decade. One of the main issues related to the increased adoption of this technology is the potential for insect resistance. In 2010, high levels of resistance to transgenic Bt corn expressing Cry1F in *S. frugiperda* larvae were reported in Puerto Rico. Using larvae acquired from corn tissue in Puerto Rico, we successfully developed strains of *S. frugiperda*. Bioassays with these larvae show high levels of resistance to transgenic Bt corn and Cry1F compared to control strains. Resistance correlated with reduced levels of alkaline phosphatase expression in the larvae. In this work we present the characterization of the mechanism responsible for field-evolved resistance to Bt corn in these *S. frugiperda* strains.

Poster / Bacteria, Monday 16:00 **B4**

Esterase activity associated with Cry1Ac-resistant *Helicoverpa zea*

Marianne P. Carey[1]; Bret Nolan[1]; Konasale Anilkumar[2]; William J. Moar[3]

[1]Case Western Reserve University, Department of Biochemistry, 10900 Euclid Ave, Cleveland, Ohio 44106, [2] Department of Entomology and Plant Pathology, Auburn University, Auburn, AL 36849; [3] Monsanto, 800 North Lindbergh Blvd, St. Louis, MO 63167

Address for correspondence: william.moar@monsanto.com

A population of bollworm, *Helicoverpa zea* (Boddie) was selected for resistance to trypsin activated Cry1Ac. Previous results showed no biologically-significant alterations in binding, a common mechanism of Bt resistance. While determining the potential for cross resistance between cypermethrin (a synthetic pyrethroid) and Cry1Ac, a significant increase in synergism was observed when Cry1Ac-resistant *H. zea* was treated with piperonyl butoxide (an inhibitor of mixed-function oxidases and esterases) in addition to Cry1Ac. Because esterases have been implicated in Bt resistance in other heliothines, esterase activity was measured in susceptible and Cry1Ac-resistant *H. zea* midgut fluids. These results will be discussed in the context of how esterase activity may interact with other potential Bt resistance mechanisms in *H. zea*.

Poster / Bacteria, Monday 16:00 **B5 STU**

Enhanced midgut regeneration as resistance mechanism to Bt toxins in *H. virescens* larvae

Anaïs S. Castagnola[1]; Omaththage P. Perera[2]; Fred Gould[3], Juan Luis Jurat-Fuentes[1]

[1]Department of Entomology and Plant Pathology, University of Tennessee, Knoxville, TN 37996, USA; [2]Southern Insect Management Research Unit, USDA-ARS, Stoneville, MS 38776, USA; [3]Department of Entomology, North Carolina State University, Raleigh, NC 27607, USA

Address for correspondence: jurat@utk.edu

One of the main issues related to the increased adoption of transgenic crops expressing *Cry* or *Vip* toxins from *Bacillus thuringiensis* (Bt) is the development of resistance in target pests. While much is known on alteration of toxin binding sites in target insects as a resistance mechanism to Cry toxins, data on alternative resistance mechanisms is limited. Considering the phasing out of single toxin Bt crops and commercialization of transgenic plants co-expressing diverse Bt toxins, our interest is focused on mechanisms resulting in resistance to diverse Cry toxins. Enhanced midgut regeneration has been previously suggested as a potential resistance mechanism to diverse Cry toxins in the KCB and CXC strains of *Heliothis virescens*. However, there is very limited data available on the regulation of midgut regeneration and significance of enhanced regeneration for resistance to Cry toxins. In this work, we demonstrate enhanced regeneration of midgut cell cultures induced by proteins secreted (secretome) by midgut cells from resistant larvae (KCB and CXC) when compared to susceptible larvae. Secretomes from resistant insects induced increased tolerance in susceptible larvae to commercial Bt products (Dipel) containing diverse toxins. Using proteomic profiling we identified proteins that may be responsible for enhanced regeneration in resistant larvae. Our research contributes to the characterization of enhanced midgut regeneration as a resistance mechanism to diverse Bt toxins.

Poster / Bacteria, Monday 16:00 **B6**

Differential Gene Expression in the Gypsy Moth (*Lymantria dispar)* Larval Midgut in response to *Bacillus thuringiensis* (Bt) infection

Michael E. Sparks; Michael Blackburn; and Dawn E. Gundersen-Rindal

USDA-ARS Invasive Insect Biocontrol and Behavior Laboratory (IIBBL), Beltsville, MD 20705

Address for correspondence: dawn.gundersen-rindal@ars.usda.gov

The gypsy moth, *Lymantria dispar*, represents a significant defoliation threat to both urban and rural regions of the eastern United States. *Bacillus thuringiensis* (Bt) is a gram-positive bacterium commonly used as a biopesticide. Here, we describe the production of a transcriptomics (RNA-Seq) dataset, delineation and functional annotation of a set of high-quality gypsy moth genes, and the larval midgut transcriptome-level response to infection by Bt *kurstaki*. An Illumina GAII sequencer was used to generate a set of ~10^8 transcript reads in wild-type and Bt-infected gypsy moth larval midgut tissue. Reads were pooled and assembled into ~50,000 Putatively Unique Transcripts (PUTs) using the Velvet/Oases short read assembler suite. BLASTx was used to compare these data to the NCBI non-redundant protein database and, according to highly stringent parsing criteria, 849 unique gypsy moth PUTs associated with 732 distinct NR proteins were identified. These high-quality genes were compared to the Pfam-A database using HMMER3 to render functional annotations using Pfam, GO and KEGG terms. To quantitatively compare wild-type and Bt-infected larval transcriptome profiles, unassembled reads were aligned to PUTs using Blat, and matches were tabulated and normalized to render digital gene expression profiles. A total of 78 gypsy moth genes were shown to exhibit at least a five-fold change in expression levels, 18 presented at least a 20-fold difference and for five, a 90-fold change or higher. These data underscore substantive differential gene expression in the gypsy moth midgut upon Bt infection, providing a foundation for identification of gene targets suitable for pest biocontrol.

Poster / Bacteria, Monday 16:00 **B7 STU**

Molecular cloning of beta-1,3-galactosyltransferase from *Helicoverpa armigera* (Hübner) and its function in relation with Bt resistance

Lili Zhang, Yanhui Lu, Qiong Lu, Gemei Liang, Kongming Wu, Yuyuan Guo

State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193, P.R. China

Address for correspondence: gmliang@ippcaas.cn

It was reported before that the nematode resistance to Bt is closely correlated with the change of beta-1,3-galactosyltransferase in its body. In this paper, beta-1,3-galactosyltransferase gene of *Helicoverpa armigera* (Hübner) was cloned and its function in relation with Bt resistance was discussed. Full length gene of beta-1,3-galactosyltransferase was cloned from the midgut of *H. armigera* by degenerative PCR combined with RACE techniques (GAL-HARM, GenBank accession No. GQ904195). GAL-HARM shared the hallmarks of beta-1,3-galactosyl transferase, including DXD and DDXYLG motif and showed high homology with that from *Bombyx mori* and *Ostrinia nubilalis*. The amino acid differences of beta-1,3-galactosyltransferase gene between resistant and susceptible *H. armigera* strain were compared. Alignment analysis demonstrated that His_{81} and Gln_{321} were respectively replaced by Asn_{81} and Thr_{321} in resistant strain. These two mutations maybe affect the function of GAL-HARM in some degree. The expression level of GAL-HARM in the midgut from Cry1Ac-resistant and -susceptible strains of *H. armigera* larvae were compared using Real-time quantitative Taqman technique. The results indicated that the expression level in Cry1Ac-resistant

strains were significantly higher than that of Cry1Ac-susceptible strain and the expression levels increased along with the raising resistance fold. The more expression of GAL-HARM in resistant strain maybe associated with resistance of *H. armigera* to Bt.

Poster / Bacteria, Monday 16:00 **B8**

Analysis on the role of *Bacillus thuringiensis* Cry toxin loop regions in binding affinity to the cadherin-like receptor using Cry toxin mutants

Yuki Fujii, Takuya Kotani, Chinatsu Morimoto, Yuko Harashima, Yasushi Hoshino, Ryoichi Sato
Graduate School of Bio-Applications and Systems Engineering, Tokyo University of Agriculture and Technology, Koganei, Tokyo 184-8588, Japan
Address and Correspondence: ryoichi@cc.tuat.ac.jp

Bacillus thuringiensis Cry toxins are used worldwide as biological pesticides with less environmental impact than conventional chemical pesticides. But Cry toxins have several demerits that they prices higher than chemical pesticides, their insecticidal spectra are narrower and so on. Therefore development of methods in which enhance or broaden Cry toxin's insecticidal activity against target pests is desired. We designed 11 classes of phage libraries that express on the phage surface Cry1Aa mutant toxins with displacement of serial 4 amino acids in any one of the toxin's 4 loop regions. Cry1Aa is toxic against the silkworm, *Bombyx mori* and we expect that Cry1Aa's insecticidal activity depends on binding affinity to a receptor, cadherin–like protein (BtR175). So, we tried selection of Cry1Aa mutants with higher-affinity to BtR175 from the phage libraries by bio-panning. We have been analyzing binding affinity of the selected mutant toxins to BtR175 using Biacore J. Our previous results suggested that Cry toxin loop regions are important for binding receptor. Meanwhile, binding affinities of all the mutant toxins which have different sequences at any one of 4 loop regions showed binding affinities ranging 0.5 to 4 fold to the wild-type toxin. This means that serial 4 amino acids displacement in any one of toxin's 4 loop regions did not lower binding affinity to BtR175 and that any sequences of toxin's 4 loop regions are not indispensable for the toxin's binding affinity. We will discuss a Cry1Aa-BtR175 binding model and important factors to insecticidal activity.

Poster / Bacteria, Monday 16:00 **B9 STU**

Binding affinity improvement of the Cry1Aa toxin to the cadherin-like protein, BtR175 from *Bombyx mori* using phage display of loop 3 mutant toxins and bio-panning

Yuki Fujii, Manami Otsuki, Yasushi Hoshino, Ryoichi Sato
Graduate School of Bio-Applications and Systems Engineering, Tokyo University of Agriculture and Technology, Koganei, Tokyo 184-8588, Japan
Address and Correspondence: ryoichi@cc.tuat.ac.jp

Development of methods that enhance or broaden Cry toxin's insecticidal activity against target pests has been being desired. In this experiment, we designed 2 types of phage libraries in which Cry1Aa mutant toxins with displacement of serial 4 amino acids in the toxin's loop 3 regions, ^{439}QAAG^{442} or ^{443}AVYT^{446} were expressed on the phage surface. Cry1Aa is toxic against the silkworm, *Bombyx mori* and Cry1Aa's activity is expected to be dependent on binding affinity to a receptor, cadherin–like protein (BtR175). So, we conducted selection of Cry1Aa mutants with higher-affinity to BtR175 from the phage libraries by bio-panning as a model for activity-improving trial for Cry toxins against insect pests. We confirmed several identical mutants had been concentrated when we analyzed gene sequences from dozens of phage clones selected by bio-panning. Therefore we analyzed binding affinity of these concentrated mutant toxins to BtR175 using Biacore J. Binding affinities of two mutants from ^{439}QAAG^{442} library and a mutant from ^{443}AVYT^{446} library were over 10 times higher than that of wild-type Cry1Aa toxin. These results suggest that loop 3 is a good region for introducing mutations to improve binding affinity of the toxin to cadherin–like protein and that phage display of the toxin is a powerful tool for the selection of mutant toxins with higher affinity to the receptor. We will discuss on the possibility that this strategy can be used to obtain activity improved or broaden mutant Cry toxins.

Poster / Bacteria, Monday 16:00 **B10**

A proteomic approach to investigate the mechanism of cross-resistance in Cry1Ab-resistant *Ostriniafurnacalis*(Guenée)

Lina Xu[1]; Natalie Ferry[2]; Zhenying Wang[1]; Jie Zhang[1]; Kanglai He[1]; Angharad M. R. Gatehouse[2]
[1]The State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, No. 2 West Yuanmingyuan Rd, Beijing 100193, China, [2]Newcastle Institute for Research on Sustainability, School of Biology, University of Newcastle, Newcastle upon Tyne, NE1 7RU, UK
Addresses for correspondence: klhe@ippcaas.cn; a.m.r.gatehouse@ncl.ac.uk

The Asian corn borer *Ostrinia furnacalis* (Guenée) (Lepidoptera: Crambidae) is the most important insect pest of maize in China causing an estimated 10-15% damage. Deployment of Bt maize is one strategy being developed to control this pest. However, under laboratory conditions it has developed more than 100-fold resistance to activated Cry1Aband readily consumes Cry1Ab-expressing maize. Although this selected strain of Cry1Ab-resistant Asian corn borerexhibits high levels of cross-resistance to Cry1Ah as well as Cry1Ac and Cry1F, no cross-resistance to Cry1Iehas been detected. Understanding the molecular mechanism of cross-resistance inAsian corn borer is thus both of theoretical and practical interest for developing effective resistance management strategies for Bt maize. A proteomic approach was exploited to identify Cry1Ab, Cry1Ah, and Cry1Ie binding proteins from the brush border membrane vesicles (BBMV) from Asian corn borer larvae. Bt binding proteins in the BBMV were detected by 2D blots and identified using MALDI-ToF/ToF mass spectrometry. A V-type proton ATPase catalytic subunit A and a heat shock 70 kDa proteins were identified as potential binding proteins for the Cry toxins tested. Surprisingly the Cry toxins showed increased binding to these protein spots in ACB-AbR BBMV when compared to ACB-BtS BBMV in ligand blots using biotinylated Cry toxins with equal amounts of protein loaded. Interestingly, Cry1Ie toxin binding to the V-type proton ATPase catalytic subunit A was not detected in ACB-BtS. The significance of these results is discussed.

Poster / Bacteria, Monday 16:00 **B11 STU**

A cadherin-like receptor mediates the in vivo toxicity of *Bacillus thuringiensis* Cry11A toxin to *Aedes aegypti*.

Su-Bum Lee[1], Jianwu Chen[2], Karlygash G. Aimanova[2], Amy M. Evans[2] and Sarjeet S. Gill[1,2]
[1]Environmental Toxicology Graduate Program, and [2]Department of Cell Biology and Neuroscience, University of California, Riverside, Riverside, CA
Address for correspondence: slee071@ucr.edu

The cadherin-like receptor is an important protein in the toxicity of *Bacillus thuringiensis* Cry proteins. We previously cloned a full-length cadherin-like receptor from *Aedes aegypti* larvae and reported this protein binds Cry11Aa toxin from *Bacillus thuringiensis* subsp. *israelensis* with high affinity, ≈ 16.7 nM. Based on these results, we investigated if the cadherin-like receptor is involved in the in vivo toxicity of Cry11A toxin to *Ae. aegypti*. First dsRNA of 507 or 781 bp were made that targeted

the *Aedes* cadherin. *Ae aegypti* larvae were then fed effectene-encapsulated dsRNA, and at early fourth instar the larvae were then treated with Cry11A at the LC_{50} level for 24 hours. Larvae that were treated with dsRNA showed decreased toxicity to Cry11A in comparison to untreated wild type larvae. This attenuation of toxicity is related to a decrease in cadherin expression observed in dsRNA-treated larvae. Next, we established a mosquito cell line stably expressing the full-length *Aedes* cadherin-like receptor. We investigated receptor expression by western blotting with a cadherin specific antibody and by immunofluorescence staining using confocal microscopy. Cell lines expressing the cadherin-like receptor were then exposed to activated Cry11A toxin. Cells expressing the cadherin-like receptor were more sensitive to the Cry11A toxin. Collectively, these results show the cadherin-like receptor plays a pivotal role in Cry11A-mediated toxicity to *Ae aegypti* larvae.

Poster / Bacteria, Monday 16:00 **B12**

A search for *Wolbachia* endosymbiont in *Agrilus* species and their associate parasitic wasps in Canada.

George Kyei-Poku and Debbie GauthierGreat Lakes Forestry Centre, Canadian Forest Service, 1219 Queen Street East, Sault Ste. Marie, Ontario, P6A 2E5, CANADA.
Address for correspondence: gkyeipok@NRCan.gc.ca

We describe surveys conducted to detect *Wolbachia* in native *Agrilus* species; *A. anxius*, *A. bilineatus* and *A. liragus* as well as their parasitic wasps that have switched hosts and may have potential for biocontrol of *A. planipennis.* The most abundant parasitoid species was *Phasgonophora sulcata* followed by *Atanycolus* spp. and the less abundant parasitoids were *Balcha indica* and *Spathius* spp. Amplification of the *Wolbachia* surface protein gene (*wsp*) detected *Wolbachia* only in populations of *P. sulcata* and demonstrated that this endosymbiont was highly prevalent (100%) in sampled populations regardless of geographic location. This high prevalence suggests an obligatory symbiosis, which may result from co-adaptation and reciprocal dependence through long coevolution of a host and its symbiont. Sequencing revealed that the *Wolbachia* harboured in *P. sulcata* populations exhibited a *wsp* belonging to a unique subgroup within the B-supergroup of known *Wolbachia*. The newly identified strain may either belong to a different strain of *Wolbachia* from those previously found to infect other arthropods or may be the result of a recombination event. We would provide information on characterization of the *P. sulcata* inherited *Wolbachia*, discuss patterns of genetic diversity in demographic history and comment on possible selective sweep and its impact on *P. sulcata* reproduction.

Poster / Bacteria, Monday 16:00 **B13 STU**

Processing of the *Bacillus thuringiensis* Vip3Aa protein by *Spodoptera frugiperda* and *S. exigua* midgut proteases

Maissa Chakroun[1,2]; Yolanda Bel[1]; Silvia Caccia[1]; Lobna Abdelkafi[2]; Baltasar Escriche[1]; Juan Ferré[1]
Department of Genetics, University of Valencia, 46100-Burjassot (Valencia), Spain[1]
Laboratory of Biopesticides, Centre of Biotechnology of Sfax, Tunisia P.O. Box ''K'', 3038, Sfax, Tunisia[2]
Address for correspondence: juan.ferre@uv.es

The Vip3Aa16 protein secreted by *Bacillus thuringiensis* BNS3 during the vegetative stage of growth displays differential toxicity towards *Spodoptera frugiperda* and *S. exigua*, with LC_{50} values of 35 (21 – 57) and 198 (79 – 804) ng/cm^2, respectively. In order to investigate the possible causes contributing to this difference, we have analyzed the involvement of midgut juice proteases of both larval species in the activation of the 90 kDa protoxin and the degradation of the 62 kDa activated toxin. The Vip3Aa protoxin, fused with a histidine tag, was purified by nickel affinity chromatography. Gut juice was collected from last instar larvae after dissection, extraction of their gut content, and centrifugation. In the case of *S. frugiperda*, the 62 kDa activated toxin accumulated with time. In contrast, in the case of *S. exigua*, the activation of the protoxin was as fast as the degradation of the toxin and, as a result, there was no accumulation of the latter. This was found no matter the concentration of *S. exigua* midgut juice was used in the assay. Because these results suggested important differences in Vip3Aa processing between species, zymograms from each one were compared. Both species differed noticeably in protease band pattern and, for the same midgut juice protein concentration, *S. frugiperda* displayed more protease bands and higher overall protease activity.

Poster / Bacteria, Monday 16:00 **B14**

A 104 kDa *Aedes aegypti* aminopeptidase N is a putative receptor of Cry11Aa toxin from *Bacillus thuringiensis* subsp. *israelensis*

Supaporn Likitvivatanavong; Jianwu Chen; Karlygash G. Aimanova; Sarjeet S. Gill
Department of Cell Biology and Neuroscience, University of California Riverside, Riverside, CA 92521 USA
Address for correspondence: sarjeet.gill@ucr.edu

The Cry11Aa protein is more toxic to *Aedes aegypti* larvae than other proteins produced in *Bacillus thuringiensis* subsp. *israelensis*, a bacterial strain used worldwide for the control of *Ae. aegypti* larvae. Cry11Aa toxin binds *Aedes aegypti* brush border membrane vesicles (BBMV) with an apparent K_d of 26 nM. Previously an aminopeptidase N (APN), named AaeAPN2, was identified as a putative Cry11Aa toxin binding protein by pull-down assays using biotinylated Cry11A toxin (Chen et al., (2009) Insect Biochem Mol Biol., 39: 688-696). Here we show this protein localizes to the apical membrane of epithelial cells in proximal and distal regions of larval caeca. The AaeAPN2 protein binds Cry11Aa with high affinity, 8.6 nM. The full-length and fragments of AaeAPN2 were cloned and expressed in *Escherichia coli*. The toxin-binding region was identified and further competitive assays demonstrated that Cry11Aa binding to BBMV was efficiently competed by the full-length AaeAPN2 and the fragments of AaeAPN2b and AaeAPN2e. In bioassays against *Ae. aegypti* larvae, the presence of full-length and a partial fragment (AaeAPN2b) of AaeAPN2 enhanced Cry11Aa larval mortality. Taken together, we conclude that AaeAPN2 is a binding protein and a functional receptor for Cry11Aa toxin.

Poster / Bacteria, Monday 16:00 **B15 STU**

Key residues of Cry5B structure and function: mutagenesis by alanine screening

Jillian Sesar, Yan Hu, Hui Fan, Partho Ghosh, and Raffi V. Aroian
University of California, San Diego, La Jolla CA 92093-0322, U.S.A
Address for correspndence: jsesar@ucsd.edu

Bacillus thruingiensis crystal (Cry) proteins are used to control insects that destroy crops and transmit human diseases. Cry proteins intoxicate invertebrates by acting as pore-forming toxins. Many defined steps in their mechanism of action have been suggested from insect studies, but there is still uncertainty as to the importance and validity of various steps.Cry proteins also intoxicate roundworms, including the common laboratory roundworm *Caenorhabditis elegans*. We believe the *C. elegans* – Cry5B system has great potential to unlock some of the mysteries surrounding Cry proteins. Here, we are investigating structure-function relationships of Cry proteins via alanine scanning. We are in the process of mutating every residue of Cry5B with alanine to determine which amino acids are important for Cry5B. What

makes *C. elegans* particularly attractive for this study is the fact that *Escherichia coli* is its standard laboratory food source. Thus, Cry5B can be directly mutated and expressed in *E. coli*, fed to the worms, and then assayed for altered properties. Here we will present our latest data from an initial screen in domain III of the protein in which we have already found mutations of interest that show both increased and decreased toxicity towards *C. elegans* as compared to the wild type Cry5B. Our ultimate goal will be to correlate these changes in activity with specific changes in protein functionality (eg, receptor biding). This study provides a way to systematically identify the important residues in Cry5B, which will provide more information on the protein function.

Poster / Bacteria, Monday 16:00 **B16**

Improving the chitinolytic activity of chitinase genes by combining them with crystal genes of *Bacillus thuringiensis*

Arzu Özgen, Kazım Sezen, Zihni Demirbağ, Remziye Nalçacıoğlu
Karadeniz Technical University, Faculty of Science, Department of Biology, 61080, Trabzon, Turkey
Address of correspondance: remziye@ktu.edu.tr

Since chitin is a major structural component of fungal cell walls and insect exoskeletons, chitinolytic enzymes could in principle be employed as natural anti-fungal agents and insect pests. In this study A chitinase producing bacterium was isolated from *Xyleborus dispar* (Scolytidae) obtained from hazelnut fields in Trabzon, Turkey. This isolate was identified as *Serretia marcescens* based on 16S rRNA sequencing results. The genes encoding *chitinase B* and *chitinase C* from *S. marcescens* amplified with dejenerate primers. Nucleotide sequences of the amplified fragments were compared with formerly identified *chitinase B* and *chitinase C* genes. The results showed that *chitinase B* and *chitinase C* genes of this isolate have 96% homologies with *S. marcescens* (BJL200) *chiB* gene and *S. marcescens* (AJ630582.1) *chiC*, respectively. In order to improve the insecticidal activity, the *chitinase B* and *C* genes from *S. marcescens* were cloned into the shuttle vector pHY300PLK, and these recombinant plasmids were introduced into *kurstaki* and *israelensis* strains of *Bacillus thuringiensis*. The insecticidal activity of engineered *Bt* strains were studied with *Galleria mellonella*, *Spodoptera littoralis* and *Drosophila melanogaster* larvaes. Engineered *B. thuringiensis* strains showed insecticidal activity higher than parental *B. thuringiensis* strains and *Serretia marcescens* isolated from *Xyleborus dispar*. The stability of plasmids was examined for engineered *B. thuringiensis* strains. Recombinant plasmids showed varied decreasing stabilities in *Bt* strains.

Poster / Bacteria, Monday 16:00 **B17 STU**

The nematocidal toxin Cry5B from *Bacillus thuringiensis* is a pore-forming toxin

Valbona Karabrahimi[1]; Cheng-Yuan Kao[2]; Hui Fan[2]; Marc Juteau[1]; Vincent Vachon[1]; Raffi Aroian[2]; Jean-Louis Schwartz[1]
[1]Groupe d'étude des protéines membranaires (GÉPROM), Université de Montréal, CP 6128, Succ. Centre-Ville, Montreal, Quebec, H3C 3J7, Canada, [2]Section of Cell and Developmental Biology, University of California, San Diego La Jolla, California 92093, USA.
Address for correspondence: jean-louis.schwartz@umontreal.ca

Cry5B is a 140-kDa protein produced by *Bacillus thuringiensis*. It is activated in *C. elegans* nematode gut into a 59-kDa toxin which elicit major morphological and cellular alterations in the host gut resulting in death within 5-6 days (LC_{50} ~ 10 µg/ml). Other Bt isolates have been shown to be active against agriculture pests and mammalian digestive tract parasites. Homology modelling shows that Cry5B toxins are members of the three-domain Bt toxin family. There is strong evidence that the toxin binds to gut cell glycolipids. Furthermore, it has been recently demonstrated that Cry5B may be responsible for host responses that may participate to bacterial virulence or induce functional defence mechanisms, including the JNK MAPK pathway and the AP1 activator protein as a downstream target of JNK MAPK protection against pore-forming toxins (Kao et al., PloS Pathogens, 7(3):e1001314, 2011).
We demonstrate here that activated Cry5B proteins form ion channels in artificial phospholipid membranes. Stable channel activity was recorded in planar lipid bilayers at 5-30 µg/ml doses. Various levels of current jumps corresponding to channel opening in response to voltage steps were recorded, with conductances ranging between 20 and 330 pS under symmetrical 150mM KCl conditions at both pH 6.0 and pH 9.5. The channels were slightly cationic (reversal potential of 11.2 mV in asymmetrical 150 mM:450 mM KCl solutions, with a permeability ration P_{K+}/P_{Cl-} = 2.5).
This is the first direct demonstration of the pore-forming capacity of a nematocidal Bt toxin. The role of the pores into nematode gut destruction, mortality and defence mechanisms remains to be clarified.

Poster / Bacteria, Monday 16:00 **B18**

Discovery and characterization of a novel *Bacillus thuringiensis* Cry1B-type insecticidal protein

Justin Lira, Joel Sheets, Stephanie Burton, and Kenneth Narva.
Dow AgroSciences, LLC, 9330 Zionsville Road, Indianapolis, IN 46268
Address for correspondence: knarva@dow.com

Despite the success of insect-resistant transgenic corn varieties based on the Cry proteins of *Bacillus thuringiensis* (Bt), the possibility of the development of resistant insect populations in the field threatens the long-term durability of commercialized Bt technology. Combining two or more Bt proteins that are not cross resistant is one strategy to prevent or delay the selection for Cry-resistant insect populations in the field. Resistance to Cry1Fa has been demonstrated in both laboratory selected colonies of *Ostrinia nubilalis* (Pereira et al. 2008) and *Spodoptera frugiperda* isolated from areas of Puerto Rico where Cry1Fa-transgenic maize was grown (Storer et al. 2010). The threat of expanding insect resistance to Cry1Fa creates the need to discover new Cry proteins with a mechanism of action different from Cry1Fa. As a class, Cry1B Bt proteins are active on a broad spectrum of lepidopteran pests (van Frankenhuyzen, 2009) and are not cross-resistant to certain other Cry1 proteins in the case of *Plutella xylostella* (Tabashnik et al. 1996; Lui et al 2001). Here we report the identification, spectrum of activity, and cross resistance assessment of a novel Cry1B-type Bt protein, DIG-3. Significantly, DIG-3 is active on Cry1A-resistant *Plutella xylostella* and Cry1F-resistant *O. nubilalis*, but is not active on *Spodoptera frugiperda*.

Poster / Bacteria, Monday 16:00 **B19 STU**

Variation of host cell components required for toxic actions of different subclasses of Cry1 toxin.

Shiho Tanaka[1]; Kazuhisa Miyamoto[2]; Hiroaki Noda[2]; and Ryoichi Sato[1]
[1]Graduate School of Bio-Applications and Systems Engineering, Tokyo University of Agriculture and Technology, Koganei, Tokyo 184-8588, Japan
[2]Institute of Insect and Animal Sciences, National Institute of Agrobiological Sciences, Tsukuba Ibaraki 305-8634, Japan
Address for correspondence: ryoichi@tuat.ac.jp

Cry toxin is the insecticidal protein toxin produced by *Bacillus thuringiensis* (*Bt*) . However, mechanism of action of the toxin may be more complicated than have been envisioned at least for some Cry1A toxins, because recently an ABC transporter class C which had never been known as a *Bt* toxin receptor was indicated

to be required for susceptivity of insects to Cry1Ab or Cry1Ac. Further, it is plausible that some Cry1 subclasses utilize specific host cell components as receptors or machineries to kill insects as have been suggested by several reports. So, we used insect cell lines, Sf9 and High Five to clarify difference of cellular components which are required for Cry1 toxins' killing activity. As a result, it was shown that both cells were originally susceptive to Cry1C but not to Cry1Aa, Cry1Ab and Cry1Ac. When BtR175, cadherin-like receptor from *Bombyx mori*, was ectopically expressed in those cells using AcNPV expression system, they became susceptive to 500 nM Cry1A toxins. These indicated these Cry1Aa, Cry1Ab or Cry1Ac toxins require cadherin-like receptor but Cry1C does not. By RT-PCR, it was indicated that both Sf9 and High Five express an ABC transporter class C. So, to know the importance of the ABC transporter class C to Cry1 toxins, we needed to knock down it or ectopically express it in the different cells which do not express it originally. We will report results of these experiments and discuss on the importance of the ABC transporter class C for Cry1 toxins' activity.

Poster / Bacteria, Monday 16:00 **B20**

Bombyx mori midgut epithelial cells' reaction induced by Cry1Aa includes both or either of osmotic swelling and apoptosis depending on toxic condition.

Shiho Tanaka; Yasutaka Yoshizawa ; and Ryoichi Sato
Graduate School of Bio-Applications and Systems Engineering, Tokyo University of Agriculture and Technology, Koganei, Tokyo 184-8588, Japan
Address for correspondence: ryoichi@tuat.ac.jp

Cry toxin is the insecticidal proteinaceous toxin produced by *Bacillus thuringiensis*. Most studies have been focused on mechanism of action of the toxin, but not of host defense. Or, in some cases, observation might have been conducted in conditions in which toxic action and host defense reaction is difficult to be differentiated**.** We examined *Bombyx mori* larval midgut which had ingested diet mixed with sublethal to lethal Cry1Aa toxin through making paraffin sections and conducting TUNEL staining to observe both toxic action and host defense reaction. When diet with lethal concentration of Cry1Aa (1.0 μg/g diet) was given to the larvae, midgut culmnar cells swelled and started to burst within 2 h, and tissues were disrupted within 24 h. In contrast, when diet with sublethal concentration of Cry1Aa (0.05 μg/g diet∼0.25 μg/g diet) which made larvae stop eating was given to the larvae, any swelling culmnar cells were not observed TUNEL highly-positive culmnar cells were detected by the staining at 4 to 24 h post ingestion, resulting in decrease of culmnar cell density in the tissue and increase of cavity space of the goblet cells. Interestinglly, when diet with medium concentration of Cry1Aa (0.5 μg/g diet) was given to the larvae, most of the cells which started to swell were observed to be TUNEL weakly-positive around 4 h post ingestion. These indicate that both or either of osmotic swelling and apoptosis can be observed depending on toxic condition in the midgut epithelium.

Poster / Bacteria, Monday 16:00 **B21**

Proteomic and genomic analysis of response to Cry intoxication in susceptible and resistant _Heliothis virescens_ larvae

Cris Oppert[1], Omaththage P. Perera[2], Fred Gould[3], and Juan L. Jurat-Fuentes[1]
[1]Department of Entomology and Plant Pathology, University of Tennessee, Knoxville, TN 37996, USA; [2]Southern Insect Management Research Unit, USDA-ARS, Stoneville, MS 38776, USA; [3]Department of Entomology, North Carolina State University, Raleigh, NC 27695, USA
Correspondence: jurat@utk.edu

Heliothine larvae are among the most economically relevant pests of several crops worldwide, and are targeted by transgenic crops expressing Cry toxins from *Bacillus thuringiensis* (Bt). New varieties of these Bt crops expressing multiple Cry toxins are being commercialized for increased range of activity and to delay the evolution of resistance to Bt crops. However, some laboratory-selected strains of *Heliothis virescens* display cross-resistance to diverse Cry toxins, highlighting the possibility that insects may develop resistance to second generation Bt crops. In an effort to understand the mechanisms involved in resistance in these strains, we performed comparative genomic and proteomic analyses of the response to Cry intoxication in susceptible (YDK) and Bt-resistant (CXC and KCB) strains of *H. virescens*. Proteins differentially expressed in response to toxin challenge were characterized using 2D differential in-gel electrophoresis (DIGE) and identified using mass spectrometry. Protein abundance patterns were compared to corresponding gene expression patterns obtained from microarrays.

Poster / Bacteria, Monday 16:00 **B22 STU**

The cleavage of the loop between alpha-3 and alpha-4 helix is critical for insecticidal activity of Cry8Da

Takuya Yamaguchi, Ken Sahara, Hisanori Bando, Shin-ichiro Asano
Department of Applied Bioscience, Graduate School of Agriculture, Hokkaido University, N9 W9, Sapporo, 060-8589, Japan
Address for correspondence: yamagu@abs.agr.hokudai.ac.jp

Larvae of Japanese beetle (*Popillia japonica* Newman) live in soil and cause severe damages to the roots of turf and vegetavles. On the other hand, adults cause damages to the leaves, flowers and fruits but the roots. Cry8Da from *Bacillus thuringiensis galleriae* SDS-502 has the toxicity against both larvae and adult Japanese beetles. Cry8Da is processed by the gut juice of Japanese beetle into three fragments (64 kDa, 54 kDa and 8 kDa). We previously reported that 54 kDa and 8 kDa fragments are derived from the cleavage of 64 kDa fragment at the loop between alpha-3 and alpha-4 helix in Domain I. Binding assay showed 54 kDa fragment bound to both larvae and adult Japanese beetle BBMV while 64 kDa and 8 kDa fragments didn't. This fact suggested that cleavage of this loop is important for BBMV binding and toxicity. In order to confirm this hypothesis, we constructed a protease-resistant mutant, Cry8Da-R163A, which is altered arginine (R^{163}) on the loop to alanine (A^{163}). This was successfully expressed in acrystalliferous *B. thuringiensis* BT51 and processed into only 64 kDa by the gut juice of Japanese beetle. This fragment, as we expected, didn't bind Japanese beetle BBMV. Also, Cry8Da-R163A didn't show significant toxicity to Japanese beetle larvae (500 μg/g), which is approximately 100 times higher LC_{50} than wild-type Cry8Da. These results strongly support our assumption that the cleavage at the loop between alpha-3 and alpha-4 helix is essential for toxicity of Cry8Da.

Poster / Bacteria, Monday 16:00 **B23**

Antimicrobial activity of bacteriocins from _Bacillus thuringiensis_ subsp. _cameroun_

Jong Bin Park[1]; Qin Liu[1]; Jae Young Choi[1]; Jae Su Kim[2] & Yeon Ho Je[1]
[1]Department of Agricultural Biotechnology, College of Agriculture & Life Sciences, Seoul National University, Seoul 151-742, Republic of Korea, [2]Research Institute for Agricultural and Life Sciences, Seoul National University, Seoul 151-742, Republic of Korea,
Address for correspondence: btrus@snu.ac.kr

Bacillus thuringiensis is characterized by its ability to synthesize crystal toxins and also able to produce bacteriocins such as thuricin, tochicin, entomocin and bacthuricin. The present work,

for the first time, describes the biological activity of bacteriocins from *B. thuringiensis* subsp. *cameroun*. Supernatant which was produced from a liquid culture of *B. thuringiensis* subsp. *cameroun* had antimicrobial activity against *Bacillus cereus*, ending up to making a clearing zone on an agar medium. A significant reduction in antimicrobial activity was observed when the supernatant was exposed to heat at 50~100°C for 15 min. Proteins were separated from the supernatant by a fast protein liquid chromatography (FPLC) given the thermal instability. A group of FPLC fractions had antimicrobial activity against *B. thuringiensis* subsp. *palmanyolensis*, *israelensis*, 1-3, *morrisoni*, *toguchini* and *kurstaki*, and *B. cereus* ACTC21768, ATCC14579 and NRRLB-569. Interestingly, when the supernatant was individually incorporated into the liquid cultures of mosquitocidal *B. thuringiensis* subsp. *israelensis* (*Bti*) and *mogi* (*Btm*), a vegetative cell growth was observed only in the *Btm* culture 10 h post-incubation. It showed a possible recovery of vegetative *Btm* cell growth compared to a control without the supernatant. These results suggest that *B. thuringiensis* subsp. *cameroun* produced proteinous antimicrobial substances, one of which may be used as a selection marker to separate *B. thuringiensis* subsp. *mogi* after conjugating the two mosquitocidal strains.

Poster / Bacteria, Monday 16:00 **B24**

Effect of *Bacillus thuringiensis berliner* in combination with tanic acid and sodium bicarbonate on caterpillars of *Gallelia mellonella* (greater wax moth)

Nouraddin Shayesteh[1], Arezo Davoudi[2], Jafar Hosseinzadeh[3]
[1]Department of Plant medicine, Mahabad Branch, Islamic Azad University, Mahabad, IRAN; [2]Department of Crop Protection, Faculty of Agriculture, Islamic Azad University, Tabriz, IRAN; [3]Department of Plant medicine, Faculty of Agriculture, Urmia University, Urmia, IRAN.
Address for correspondence: shayesteh@yahoo.com

In this study, caterpillars of Greater wax moth were collected out of the honey bees of west Azarbayezan and the they were cultured on old black wax for 1.5 years under 27 ± 2°C and 45 ±5 percent relative humidity and different doses of three serotypes hamely (Kurstaki, Thuringiensis, Galleria) were tested on them employing two methods of Spray and Powder and each test was performed in the form of an absolutely random pattern with three repetition and seven treatments using 45 caterpillars in each repetition.Having determined the death rate in each repetition and using probite analysis, their LC_{50} was calculated and comparediwe found that Kurstaki serotype with 415.049 pp.m LC_{50} with Spray Method and 734.26 ppm with powder method has had the best effect. In this study, tanic acid and baking soda (Sodium bicarbonate) in combination with low doses of Kurstaki Variety with Spray method were used as strengthening substance, the LC_{50} with probit analysis method and test with powder Method for tanic acid has been evaluated 22740.027 ppm and for baking soda 106.000.000 ppm. It was revealed that tanic acid with 12000 ppm which causes 48.9% death increases its killing property in combination with bacteria especially in 50.112.570ppm at 2.1, 4.4, and 2.1 percent respectively. In the study of determining the effect of feeding time, it has been revealed that 2, 15.5 and 72 hours, the death rate for the two instarts larval have been 10.50 and 73% respectively. According to the tests for the effect of age and time with factorial method and the death rate of different instarts of 1,3,5,7 and 9 caterpillars in the samples independent of time and sampling time independent of age. It was revealed that there is significant difference between them. As a result of accompaning age with time variables, it was clear that the older the caterpillars get and increased the installs, the less. The sensitive of caterpillars to disease generating bacteria will be come, and the death rate of caterpillars with instarts 1,3 and 5 will be in on row after 24,48,and 72 hours respectively and the death rate of the caterpillars instars 7 and 9 will be in one row after 72 and 96 hours. According to the results of study, it is concluded that the doses lower than 500 ppm from Kurstaki bacteria in combination with tanic acid and baking soda (Sodium bicarbonate) in powder from mixed with ground wax has the maximum effect according to the behaviours of the low instars caterpillars which often live on the hives floors, the control of caterpillars of the wax-eating larval is more practical, more economical in comparison with other methods if the after-menticned substances are used on hives roofs in the from of mixture with ground wax.

Poster / Bacteria, Monday 16:00 **B25**

From non-target risk assessment to Bt resistance management: the example of Bt *Brassica* sp. and *Pieridae*

Barbara Manachini[1], Filippo Castiglia[2]
[1]Dep. Environmental biology and Biodiversity, University of Palermo, Via Archirafi 18, 90123 Palermo. Italy, [2]Dipartimento Regionale Azienda Regionale Foreste Demaniali Servizio 3° Servizio gestione delle aree protette (UOB n. 1) Via Libertà, 97 Palermo. Italy.
Address for correspondence: b.manachini@unipa.it

Insecticidal toxins produced by *Bacillus thuringiensis* (Bt) have been widely used in plant protection in two different ways: 1) by spraying bacterial Bt formulations, 2) by expressing their gene encoding *in planta*. In the second strategy, only the transgenic plants themselves are protected but insect- resistant plants could spread into natural habitats and/or the gene could be fixed in alternative hosts. It is necessary to estimate the magnitude of the possible effects on naturally occurring herbivorous insects but also on the target species, especially when they have several alternative hosts, as in the case of Pieridae. They are present both in agriculture where they could be considered pests, and non-agricultural habitats, where the larvae feed on several Brassicaceae. Moreover, oilseed rape is known to form feral populations in natural and semi-natural habitats, and that Bt-oilseed rape may out-compete insect-susceptible plants at high herbivore densities. Furthermore, high-density patches of highly damaged wild plants are the most vulnerable to *Bt*-transgene invasion. To assess the potential risk of Bt resistance in Pieridae, different parameters should be considered. In this contest we thought it worthwhile to study the population density of Pieridae, their fly period compared with the flowering period of potential Bt crops and relative wild species and its hosts preferences Moreover we report data on susceptibility to commercial Bt and to CryIAb toxin. Such data may both aid the design of further tests of related effects and aid in the assessment of any effects on the population outside the agricultural area.

Poster / Bacteria, Monday 16:00 **B26**

Development of a PCR assay and DNA probe for the detection of *Pseudomonas fluorescens* in naturally infected Nile tilapia (*Oreochromis niloticus*, L.) tissues.

Medhat. H. Hashem[1], Omaima A. Khamiss[1], , Elsayed A. Khallaf[2], Adell A. Guirgis[1], and Khalil A. EL Halafawy[1].
[1]Genetic Engineering and Biotechnology Research Institute (GEBRI), Minufiya University.
[2]Faculty of Science - Minufiya University

Pseudomonas fluorescens was isolated from Nile tilapia *Oreochromis niloticus* fish. Three concentrations of $4x10^{11}$, $4x10^{8}$ and $4x10^{5}$ cells/ml were used to study the pathogenicity of bacteria. The percentage of cumulative mortality in the *O. niloticua* fingerlings was 76.7, 60 and 36.6%, respectively and the calculated LD_{50} was $4\ X10^{7}$cells/ml. Polymerase chain reaction and DNA probe were used in diagnosis of *P. fluorescens* by testing specific primers of bacterial 16S rRNA gene, different annealing temperatures and different types of samples (purified DNA, and different concentrations of diseased fish tissue homogenates). The specific

primers gave a unique and specific amplification band in an 848 base pair lengths at different annealing temperature. DNA specific probe was tested with internal organs of diseased *O. niloticus* homogenates and purified DNA of *P. fluorescens* and positive result was obtained with all samples.

Poster Papers Monday, 16:30-18:30

Microbial Control

Poster / Microbial Control, Monday 16:00 **MC1 STU**

Mycosis at cool temperatures: the susceptibility of the temperate grape cutworm *Abagrotis orbis* to entomopathogenic fungi

Scott Johnson[1], Tom Lowery[2], Jenny Cory[1] and Joan Cossentine[2]
[1]Department of Biological Sciences, Simon Fraser University, 8888 University Drive. Burnaby, BC, V5A 1S6 Canada, [2]AAFC, Pacific Agriculture Research Centre, 4200 Highway 97, Summerland, BC V0H 1Z0 Canada.
Address for correspondence: tsjohnso@sfu.ca

Abagrotis orbis (Grote) (Lepidoptera: Noctuidae) is a common climbing cutworm in the temperate vineyards of western North America. Eggs deposited in the fall develop to later instar larvae that feed on grapevine buds in the spring; consequently much of their active feeding periods occur under cooler temperatures. The cutworms have an intimate relationship with the vineyard floor spending most of their lives in soil or under debris on the soil surface. We sought to evaluate the level of their susceptibility to *Beauveria bassiana* isolate GHA and *Metarhizium anisopliae* isolate F52 at 10, 15 and 20 ^{o}C under laboratory conditions. All treatments were made as 24 h exposures of *A. orbis* larvae to *Brassica rapa chinensis* leaf discs, dried after immersion in a range of conidia concentrations. As hypothesized, *A. orbis* larvae were found to be susceptible to both entomopathogenic fungi with the highest mortality rates occurring at 20 ^{o}C. Significant mycosis however was found to take place at the lower temperatures of 15 and 10 ^{o}C and was a function of instar.

Poster / Microbial Control, Monday 16:00 **MC2**

Adaptive melanism and immunity to fungal infection in the migratory grasshopper

Robert B. Srygley and Stefan T. Jaronski
USDA-Agricultural Research Service, Northern Plains Agricultural Research Laboratory, 1500 N. Central Ave., Sidney MT 59270 USA
Address for correspondence: Robert.Srygley@ars.usda.gov

Is decreased melanism in many grasshoppers, in response to higher environmental temperatures, associated with less enzymatic immunity and greater vulnerability to fungal attack, or not? *Melanoplus sanguinipes* were reared from the third instar to adult at either 39° C or 28° C. We assayed circulating phenoloxidase (PO) and prophenoloxidase (proPO) activity in the blood of adults of precisely known age. We also assayed survival to attack by *Beauveria bassiana* strain GHA by topically applying a dose of $1*10^5$ conidia / µl oil per insect), with oil alone as a control, and followed survivorship for 14 days. Grasshoppers reared in the hot environment were larger and lighter in color than those reared at the cool temperature. Blood PO and proPO titers of those reared in the hot environment were significantly less than those reared in the cool one. Although rearing temperature did not have a significant effect on survivorship of the controls, it did affect survivorship of fungus treated grasshoppers. Consistent with differences in PO and proPO activities, those reared in the hot environment had greater mortality from fungal infection and shorter median survival time relative to those reared at the lower temperature. Our data indicate that melanism in response to ambient temperature can also be associated with changes immunity and the ability to defend against fungal attack.

Poster / Microbial Control, Monday 16:00 **MC3**

Storage of *Metarhizium anisopliae* at low temperature on rice substrate aiming sugar cane pest control

João R. Alencar[1]; Ricardo A. Polanczyk[1]; Marina A. Viana[2]; Sergio A. De Bortoli[2]; Thiago T. Agostini[1]; Alessandra M. Vacari[2]; Jaqueline M. Maeda[2]
[1]Laboratory of Arthropod Pests Microbial Control, Universidade Estadual Paulista. Access route Paulo Donatto Castellane, Jaboticabal, SP 14884-900 Brazil. [2] Laboratory of Rearing and Biology of Insects, Universidade Estadual Paulista. Access route Paul Donata Castellane, Jaboticabal, SP 14884-900 Brazil
Address for correspondence: rapolanc@fcav.unesp.br

Metarhizium anisopliae is widely used in Brazil to control pests in various cultures, mainly sugar cane pests. There are a lot of medium for mass production of this entomopathogenic fungi, and the most use in Brazil is the parboiled rice medium. The objective was to evaluate the viability of *Metarhizium anisopliae* at low temperature storage to measure the impact of storage on efficacy of the fungi in the field. The entomopathogenic fungi was cultivated in a medium composed by grains of parboiled rice sterilized in an autoclave. After growth of *M. anisopliae* in the medium, it was kept stored in a climate chamber at -4 º C. Weekly evaluations were done by suspending five samples (constituting the treatment replicates) of 5 g of material in 5 mL of deionized water and autoclaved. The evaluations were done by counting the conidia in Neubauer chamber, over time. The average conidia per gram of culture medium decreased with values ranging from of 5.57×10^9 conidia.g^{-1} on 14 September 2010 to 7.19×10^8 conidia.g^{-1} on April 5 2011, a reduction of approximately 7.75 times the initial concentration. The conidial mean was differed at each evaluation and regression analysis resulted the quadratic equation $y=299.170.331.905.692-14.733.755.781,90\ x+181405.01x^2$ and $R^2=0.98$. Even in cold storage, there is a quantitative decrease in the number of conidia present in the culture medium. Therefore, it is recommended that quantify the samples just prior use, to prepare the suspension at appropriate concentration.

Poster / Microbial Control, Monday 16:00 **MC4**

Association of entomopathogenic fungi with exotic red palm weevil in treated and untreated *Phoenix canariensis*

Barbara Manachini, Franco Palla
Department of Environmental Biology and Biodiversity, University of Palermo, Via Archirafi 38, 90123 Palermo, Italy
Address for correspondence: b.manachini@unipa.it

Rhynchophorus ferrugineus (Olivier) (Coleoptera: Curculionidae) is introduced pests of established *Phoenix canariensis* in Sicily (Italy). A three-year field survey of *R. ferrugineus* generated data on species of fungal associated with larval, pupae, and adult *R. ferrugineus* recovered from several cadavers. Moreover specimens were collected from infested *Phoenix canariensis* in scattered locales in Sicily to determine also infection rates with entomopathogenic fungi over 3 years. Collection was done after overwintering period, during summer and at the beginning of autumn in palm, untreated and treated with chemical pesticides. As can be expected the highest infestation was recorded after overwintering period in untreated palms. The infection rates of larvae, pupae, males, and females were 5.0%, 35.0%, 3.5% and 4.3% respectively. Samples mainly from field-collected pupae, were utilized to inoculate Nutrien agar plate. Isolated fungal colonies were characterized both by scanning (S.E.M.) and confocal laser scanning (C.L.S.M.) microscopy and molecular analysis (specific molecular markers). Entomopathogenic fungi were identified as *Beauveria bassiana*, *Pichia* sp., *Trichosporon multisporum* and *Trichosporum* sp., *Verticillium lecanii* and *Verticillium* sp. (a member of the Verticillium lecanii species complex).

Poster / Microbial Control, Monday 16:00 **MC5**

Molecular characterization and virulence of *Beauveria* isolates from emerald ash borer within old outbreak sites in Canada

Shajahan Johny; George Kyei-Poku; Kees van FrankenhuyzenCanadian Forestry Service, Great Lakes Forestry Centre, 1219 Queen Street East, Sault Ste. Marie, Ontario, Canada P6A 2E5.
Address for correspondence: gkyeipok@NRCan.gc.ca

The emerald ash borer, *Agrilus planipennis* (EAB), is an invasive wood boring beetle that is decimating North America's ash trees (*Fraxinus* spp.). To date, an estimated 30 million ash trees have succumbed to EAB infestation. The current rapid expansion of EAB poses a substantial risk to the remaining ash resources of North America. To find effective and safe indigenous biocontrol agents, we conducted a survey to recover entomopathogenic fungi (EPF) infecting EAB from old outbreak sites within the Province of Ontario, Canada. In each site, mycosed cadavers, larval gallery frass, soil, ash bark, and live beetles were sampled. A number of isolates were cultured from the targeted samples and subsequently characterized with ribosomal internal transcribed spacer (ITS) rDNA fragment. The analysis revealed that the genus *Beauveria* was the most common fungi species associated with EAB and its habitat. The EAB-recovered *Beauveria* isolates were further characterized with two nuclear intergenic regions, B-locus and elongation factor 1-alpha (EF1-α). Based on their clustering on a phylogenetic scale, twenty-five of the EAB-recovered *Beauveria* isolates were selected and check for their pathogenicity and virulence against EAB using a single concentration, 2.0 x 10^7conidia/ml. The five most virulent EAB-recovered *Beauveria* isolates were compared with the commercial *Beauveria bassiana* strain, GHA at four different concentrations viz., 2.0×10^4, 2.0×10^5, 2.0×10^6, and 2.0 x 10^7conidia/ml. Virulence of three local isolates viz., L491AA, L11A, and L19C were comparable to the commercial isolate GHA. Isolates L491AA, L11A and L19C also produce more conidia both in vitro and on mycosed EAB than GHA, suggesting that these local isolates have better qualities and can be used to manage EAB infestations.

Poster / Microbial Control, Monday 16:00 **MC6**

Characterization, pathogenicity and virulence of EAB-recovered *Isaria* and *Paecilomyces* isolates.

Shajahan Johny; George Kyei-Poku; Kees van FrankenhuyzenCanadian Forestry Service, Great Lakes Forestry Centre, 1219 Queen Street East, Sault Ste. Marie, Ontario, Canada P6A 2E5
Address for correspondence: gkyeipok@NRCan.gc.ca

Entomopathogenic fungi of the genera *Isaria* and *Paecilomyces* were recovered from old outbreak populations of emerald ash borer in Southern Ontario, Canada. We identified and characterized these fungal isolates as *Isaria farinosa* and *Paecilomyces lilacinus* based on morphological criterion. We also performed a molecular phylogeny on these taxa, based on ITS1-5.8S-ITS2 rDNA, partial SSU rRNA and partial β-tubulin gene sequences. Our phylogenetic analysis and constructed trees based on the SSU rRNA, ITS and tubulin genes clearly separate the *Isaria* and *Paecilomyces* into two distinct groups and explicitly confirm isolates L66B-A, SY17-A and LHY46-A as *I. farinosa* and isolates B3A, B59A and SY45B-A as *Paecilomyces lilacinus*. Pathogenicity of the EAB-recovered *I. farinosa* and *P. lilacinus* was tested against EAB using a single concentration, 2.0 x 10^7conidia/ml. Control isolates included three commercial isolates namely; *Isaria* (*Paecilomyces*) *fumosoroseus* (PFR-97), *Metarhizium brunneum* (F-52) and *Beauveria bassiana* strain, GHA. The results showed that the EAB-recovered *I. farinosa* was less virulent compared to the commercial isolates but yearly isolation from EAB populations suggests it is one of the natural control agents of EAB in Canada.

Poster / Microbial Control, Monday 16:00 **MC7**

Influence of fungicides on spore viability and pathogenicity of *B. bassiana* GHA

Jeong Jun Kim[1], Hong Zhu[1], Yujin Song[1], Yunheui Park[1], Manyoung Choi[2], Sang-Guei Lee[2], Jaehong Yoo, and Sangyeob Lee
[1]Agricultural Microbiology Team, NAAS, RDA, Korea, [2]Division of Crop Protection, NAAS, RDA, Korea
Addresses for correspondence: jjkim66@korea.kr; jjkim66@hotmail.com

Concerns regarding entomopathogenic fungi as alternative pest control agents are increasing even though chemical pesticides have been used as the main control agents for pests and diseases in crop production. This study was conducted to test the influence of fungicides on *Beauveria bassiana* isolate GHA. Among the 11 tested fungicides which are commonly used fungicides in Korea, copper hydroxide and ethaboxam did not inhibit spore germination and mycelial growth of *B. bassiana* at various concentrations. Also, the time of tank mixture between GHA and fungicide and the fungicide concentration did not influence spore viability. Fungicides were applied to potted cucumber plants 7, 4, 1, and 0 days before GHA application. When both GHA and fungicides were applied at the same day, mortality in whiteflies 2 days after treatment was 30%, 24%, 15%, 23%, 22%, 24%, and 15% for Botanigard only, copper hydroxide, dimethomorph, fenarimol, propineb, tetraconazole, and trifulmizol, respectively. Spray intervals of 7 and 4 days between fungicides treatment and GHA application appeared to have no deleterious effects on the whitefly control**.** These results demonstrated that with careful selection and timing of fungicide applications can make them compatible with microbial control agents in integrated pest control.

Poster / Microbial Control, Monday 16:00 **MC8**

Entomopathogenic fungi in sugar maple forest soils

B.L. Parker, M. Skinner, S.Y. Gouli, V.V. Gouli, J-S. Kim, D. Tobi, and Ch. Frank
Entomology Research Laboratory, University of Vermont, 661 Spear Street,
Burlington VT 05405-0105, USA
Address for correspondence: svetlana.gouli@uvm.edu

Numerous invertebrate pests are periodically or permanently found in forest soil especially during pupation or (and) overwintering. An important pest of sugar maple trees, pear thrips, *Taeniothrips inconsequens* (Uzel), remains in the soil for ~10 months/y. This insect periodically causes significant foliage damage in Northeastern US forests. Three areas in Underhill, VT were selected for the study. Two areas contained Lyman-Marlow very rocky loam soil and the third Peru stony loam soil type. Ninety nine soil plots were randomly selected where pear thrips are known to pupate (Skinner and Parker, 1991). Analyses of soil samples showed the presence of *Beauveria bassiana (Bb)*, *Metarhizium anisopliae (Ma)*, *Isaria farinosus*, *I. fumosoroseus (I*spp*)*, *Paecilomyces lilacinus (Pl)*, *Lecanicillium sp. (L*sp*)* and *Conidiobolus coronatus (Cc).* Fungi *P*spp and *L*sp were the most common in all areas. There were plots that contained 63% to 82% of *P*spp and 24% to 85% of *L*sp. The first area contained the largest number of *L*sp (96.9%) compared to the second (69.7%) and the third (9%). Species from the genera *Isaria* and *Paecilomyces* were distributed more evenly across three stands. The plots with the most *P*spp fungi (81.8%) were found in the second area. The first and third contained 43.4% and 72.7% *P*spp fungi respectively. Fungi *Bb* and *Ma* were distributed randomly in different areas. The first and second areas contained 12.1% and 6% plots with *Bb* respectively, but the third contained 75.7%. A similar situation was observed in case of *Ma* which was found only

in plots from the second area, and significantly more in the first (36.3%) and the third (57.5%) areas. Fungus *L*sp and fungi from the genera *Isaria* and *Paecilomyces* were isolated together in the same plots in all three areas: the first contained 8 (24.2%) such plots, the second – 18 (54.5%), and third – 1 (3%). The third area contained 14 plots (42.4%) with *Bb*, *Ma* and *P*spp. There is a correlation between *Bb* and *L*sp fungi: when the first fungus predominates, the second is seldom seen, and vise versa. For example, in case of the first area, 32 plots contained *L*sp and only 4 plots contained *Bb*. A diametrically opposite situation was found in the third area where *Bb* was in 25 plots and *L*sp in 3 plots. Calculation of the number of fungal propagules per one gram dry soil showed the following: the first area contained *Bb* propagules between $2x10^3$ and $6x10^3$, *Ma* between $2x10^3$ and $1.4x10^3$, *L*sp between $2x10^3$ and $1x10^4$, *P*spp between $2x10^3$ and $1{,}6x10^4$ and *Cc.* between $4x10^3$ and $1.2x10^4$.; the second area contained *Bb* propagules between $2x10^3$ and $2x10^4$, *Ma* $2x10^3$, *L*sp between $2x10^3$ and $7x10^3$, and *P*spp between $2x10^3$ and $3x10^4$.; the third contained *Bb* propagules between $2x10^3$ and $2x10^4$, *Ma* $1x10^4$, *L*sp $2x10^3$, and *P*spp between $2x10^3$ and $1.6x10^4$. During the period of soil sampling there was no damage to sugar maple trees by pear thrips.

Poster / Microbial Control, Monday 16:00 **MC9**

Are *Beauveria bassiana* treatments appropriate for orchard canopies?

Joan Cossentine and Paul Randall
AAFC, Pacific Agri-Food Research Centre, 4200 Highway 97, Summerland, BC V0H 1Z0 Canada.
Address for correspondence: joan.cossentine@agr.gc.ca

Leafrollers are common secondary pests in temperate fruit orchards that can benefit from significant natural parasitism. *Beauveria bassiana* (Balsamo) Vuillemin formulations are being considered as potential orchard canopy treatments for other orchard pests and laboratory trials were conducted to evaluate the susceptibility of immature obliquebanded leafrollers, *Choristoneura rosaceana* (Harris) to *B. bassiana*-GHA. Under ideal conditions, egg masses $\leq$ 24 h old were susceptible to infection by topically sprayed *B. bassiana* conidia and the resulting mycosis significantly reduced the number of neonates emerging from treated eggs. Laboratory exposure of young larvae to *B. bassiana*-GHA on apple leaf discs caused significant dose-related mortality in first to fourth instars. First instar *C. rosaceana* were the most susceptible of the larval stages with 80 % of the neonates becoming infected and dying from exposure to leaf discs treated with 1×10^8 conidia ml^{-1}. Of the second to fourth instars however, only the latter exhibited mortality >50% after exposure to leaf discs treated with the relatively high dose of 1×10^8 conidia ml^{-1}. Both *C. rosaceana* egg masses and early instars are susceptible to parasitism in orchards with reduced or no chemical pesticide usage. The impact of *B. bassiana* treatments on *C. rosaceana* parasitoids was tested under laboratory conditions. The benefits and risks of potentially low host *B. bassiana* suppression in the field and the impact that the pathogen may have on non-targets are considered.

Poster / Microbial Control, Monday 16:00 **MC10 STU**

Biopesticides for the control of greenhouse whitefly in Australia.

Spinner, JE, Wilson, B, Stodart, BJ, Hauxwell, C, Ash, GJ.
EH Graham Centre for Agricultural Innovation (Charles Sturt University and Industry & Investment NSW), Boorooma Street, Wagga Wagga, NSW 2678.
Address for correspondence: jspinner@csu.edu.au

The greenhouse whitefly (GHWF), *Trialeurodes vaporariorum* (Westwood) (Homoptera: Aleyrodidae), is a key pest of glasshouse crops around the world. Entomopathogenic fungi have been used to improve the management of whiteflies overseas, but in Australia no fungal biopesticides have been registered for this pest. Due to Australia's strict quarantine laws, it is less problematic to develop native isolates into registered products than to import isolates. Two species of fungi, *Beauveria bassiana* (Balsamo) Vuillemin and *Paecilomyces* spp. Samson (Deuteromycota: Hypocreales) were selected from the collection of the Queensland Department of Primary Industries. Molecular characterisation using internal transcribed spacers confirmed the identification of the *B. bassiana* isolates and classified the *Paecilomyces* spp. as *Isaria fumosorosea* (Wise). The pathogenicity and virulence of these native Australian isolates was tested to determine the most useful isolate of each species against GHWF and compared with the overseas commercial strains (Mycotrol®; Laverlam and PFR-97®; Certis) in on-plant assays. Future work will examine the effect of the entomopathogens on predators and parasitoids of whitefly as well as the effect on other pest insects of greenhouse tomatoes. Interactions with the parasitic wasp *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae) under Australian conditions will be examined as a model system.

Poster / Microbial Control, Monday 16:00 **MC11**

Control of grain pests using *Beauveria bassiana* combined with an electrostatic powder (Entostat®).

Clare Storm[1], Maureen E. Wakefield[2], Dave Moore[3], Belinda Luke[3], Bryony Taylor[3], Pierre Grammare[4], Oliver Poitin[4] and Stephen Abolins[1]
[1] Exosect Ltd, Leylands Business Park, Colden Common, Winchester, SO21 1TH, UK, [2] The Food and Environment Research Agency, Sand Hutton, York, YO41 1LZ, UK, [3] CABI Europe-UK, Bakeham Lane, Egham, Surrey, TW20 9TY, UK, [4] Sylvan Bio – Europe, Zi Tivoli, Route de Mauvieres, 37600 Loches, France
Address for correspondence: stephen.abolins@exosect.com

Stored product pests can cause serious damage to grain, leading to large losses in yield. Traditionally, control has been achieved through the use of chemical pesticides such as the organophosphates. However, due to problems such as resistance of the pest, concerns over residues and product taint as well as the withdrawal of some chemical treatments, novel methods of control are needed. Many new substances have been tested for their potential to control grain pests, such as diatomaceous earth, entomopathogenic fungi and introduction of natural predators. However none of these methods have been able demonstrate the levels of control needed to be viable treatments at a commercial scale. The work presented here will demonstrate the potential of an electrostatic powder (Entostat®) combined with *Beauveria bassiana* (Balsamo) Vuillemin as a treatment against several species of grain pest. Due to the unique colocation of the Entostat® particles with conidia of *B. bassiana*, forming a composite particle, high levels of mortality have been achieved under a variety of conditions with the potential to create a product that can be used as a treatment for the building fabric and also as an admixture in grain.

Poster / Microbial Control, Monday 16:00 **MC12**

Biological control of stem borer,*Chilo partellus* Swinhoe through *Trichogramma chilonis* Ishii and insect pathogens in Sorghum

Shekharappa
AICSIP, University of Agricultural Sciences, Dharwad-580 005: India
Address for correspondence: shekhar1993@yahoo.com

The experiment was conducted to manage the stemborer, *Chilo partellus* Swinhoe by utilisinng egg parasitoid, *Trichogramma*

chilonis Ishii and insect pathogens viz.,*Bacillus thuringiensis* and *Beauveria bassiana* in Sorghum at University of Agricultural Sciences, Dharwad.The extent of parasitisation of stemborer eggs was as high as 63.33%. The release of *T. chilonis* @1 lakh/ha five times followed by *B. thuringiensis*@1gm/l spray(one sray in each generation after five days of appearance of larva) twice caused 85.60% larval mortality. Similarly, *B bassiana* @1gm/l twice application had registered 70.65% larval mortality. The integration of *T. chilonis* release followed by sprayieng of microbial insecticides viz., *B. thuringiensis* and *B bassiana* minimum damage of pinholes,dead heart,peduncle damage and stem tunneling due to stem borer, *C partellus* and maximum yield which was on par with standard check viz., NSKE@5% and Endosulfan (0.07%). Microbial insecticides individual application two times also proved their superiority over other treatments and on par with standard check.

Poster / Microbial Control, Monday 16:00 **MC13**

Effectiveness of a granulovirus and *Bacillus thuringiensis* strains on *Cydia pomonella* in laboratory and orchards

Xiangyang Liu, Chengfeng Lei and Xiulian Sun
Key Laboratory of Agricultural and Environmental Microbiology, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan 430071, China
Address for correspondence: sunxl@wh.iov.cn

The codling moth *Cydia pomonella* is one of the most serious pests of apples worldwide. In this study, a *C. pomonella* granulovirus (CpGV) isolate (CpGV-C) was obtained from *C. pomonella* cadavers collected in an orchard in Gansu, China. Droplet-feeding bioassays showed that the median lethal concentration (LC_{50}) of CpGV-C against third instar *C. pomonella* larvae was 7.7×10^5 OBs/ml at ninth day post inoculation. The LC_{50} values of *Bacillus thuringiensis* (Bt) C-33 and kurstaki HD-1 were 26.3 µg/ml and 15.7 µg/ml, respectively. Orchard tests indicated that control efficacies of CpGV and combination of CpGV and Bt at 1:15 were similar to that of beta-cypermethrin. Our data provide a valuable reference for the application of CpGV or combination of CpGV and Bt in controlling *C. pomonella* under field conditions.

Poster / Microbial Control, Monday 16:00 **MC14**

Gene transfer by densovirus derived vectors for biological control

Aurélie Perrin[1,2], Cecilia Multeau[1,2], François Cousserans[2], Max Bergoin[2], Philippe Fournier[2] & Mylène Ogliastro[2]
[1] Union Invivo-Biotop, 83 avenue de la Grande Armée - 75782 Paris Cedex 16, [2] Unité DGIMI, UMR 1333 INRA - Université Montpellier II, Place Eugène Bataillon, Bât. 24, cc101, 34095 Montpellier Cedex 5
Address for correspondence: perrinau@supagro.inra.fr

Densoviruses (DNVs) are small icosahedral non-enveloped viruses, containing a single-stranded linear DNA genome, they belong to *Parvoviridae* family. DNVs are highly infectious for insects and crustacean (Tijssen & Bergoin, 1995). Field trials have shown their potential interest as biological control agents, however the time to death of an infected host is quite long and DNVs host range is poorly documented (Genty & Mariau, 1975). We developed a new strategy for biological control based on the *Junonia coenia* Densovirus derived vectors. *Jc*DNV has an ambisens genome of 5996 bp with two complementary strands. One strand carries three open reading frame (ORF2 to ORF4) encoding the 3 nonstructural proteins (NS1 to NS3) expressed under control of P93 promoter. The complementary strand has one large ORF1 encoding 4 structural proteins (VP1 to VP4) expressed under control of P9 promoter. Long Inverted terminal repeats, ITRs, are present at the extremities of each strands and are involved in DNA replication and genome encapsidation. The aim of this project is to produce DNV non replicative "toxic" particles in order to kill faster and prevent unwanted dispersal. Recombinant JcDNV pseudo-particles were produced by multi-transfection of permissive Ld cells, with 3 *Jc*DNV constructs separately carrying the NS, the VP and the recombinant genome driving the expression of a reporter gene. Preliminary results showed that recombinant Densovirus pseudo-particles were reconstituted in insect cells and these particles allowed the delivery of reporter genes in insects (*Spodoptera* sp.). These pseudo-particles did not replicate in target tissues.

Poster / Microbial Control, Monday 16:00 **MC15**

A potential bacterial control agent against cotton leaf worm (*Spodoptera littoralis,* Lep.: Noctuidae)

Filiz Ozkan, Zihni Demirbag, Ismail Demir
Department of Biology, Faculty of Sciences, Karadeniz Technical University, 61080, Trabzon, Turkey
Address for Correspondence: zihni@ktu.edu.tr

Spodoptera littoralis, is one of the most destructive agricultural lepidopteron pests within its subtropical and tropical range, has important damages on plants belonging to 44 different families including grasses, legumes, crucifers and deciduous fruit trees. Although various methods are being used to control this pest, its damage still continues effectively all over the world. In order to find a significant microbial control agent against this pest, first of all we determined 10 bacterial isolates and identified these isolates at species level based on morphological, physiological, biochemical and molecular characters. The insecticidal effects of 10 bacterial isolates from *S. littoralis* and 12 bacteria belonging to *Bacillus* genus from our culture collection were tested on the 3rd instar larvae of the pest. Bacterial isolates from *Bacillus* genus with codes FOTRB 6 (from *Malacosama neustria*) and FOTRB 11 (from *Balanninus nucum*) have 100% mortality on the pest within 10 days. The *Bacillus* species FOTRB 6 from our culture collection was studied more detail. It was detected that this isolate has 97%, 27%, 100% and 97% mortality effects on 1., 2., 3. and 4. instar larvae of the pest within 10 days, respectively. Flowerpot assay showed that this isolate has an important effect on the pest at laboratory conditions. In the further research, development of a suitable formulation from this isolate and its application against *S. littoralis* is planned.

Poster / Microbial Control, Monday 16:00 **MC16**

Preliminary results on antimicrobial activity of *Rhynchophorous ferrugineus* hemolymph

Domenico Schillaci[1], Francesca Tiziana Giaramita[2], Barbara Manachini[2], Vincenzo Arizza[2]
[1]Dep. Stembio, University of Palermo, Via Archirafi 32, 90123 Palermo Italy, [2]Dep. Environmental biology and Biodiversity, University of Palermo, Via Archirafi 18, 90123 Palermo Italy
Address for correspondence: dschill@unipa.i

Rhynchophorus ferrugineus (Coleoptera: Curculionidae), known as the Red Palm Weevil (RPW) and accidentally introduced in the Mediterranean areas, is considered a quarantine pest for tree palms especially in the urban environment. In order to understand the relationship among potential pathogens and RPW several studies were carried out on RPW immune system. Here we report the results of a preliminary study undertaken to investigate the effect of naturally occurring antimicrobial activities present both in the cell free hemolymph and in hemocytes lysate supernatant of RPW. The hemolymph was extracted from the older larvae sampled from infested palm trees. The protein fractions were extracted from hemolymph and hemocyte lysate supernatant by acidic precipitation. Antimicrobial activity was tested against a group of medical, veterinary and entomo-pathogenic bacterial strains. The antimicrobial activity was evaluated by determining minimal

inhibitory concentrations (MICs) against planktonic form of the isolates using a standard micro-method. Data from this research could be useful to help for screening selection of entomopathogenic as well to search potential antimicrobial peptides against human or veterinary pathogens from insect sources.

Poster / Microbial Control, Monday 16:00 **MC17**

Anti-fungal activity of *Bacillus thuringiensis* Delta-endotoxin towards plant pathogenic fungi of *Phytophthora* and *Fusarium* genera

L. K. Kamenek[1], D. V. Kamenek[1], A. A. Tyulpinyova[1], M. A. Terpilowski[1], V.V. Gouli[2]
[1]Ulyanovsk State University, 42 Tolstoy Street, Ulyanovsk, 432700, Russia; [2]University of Vermont, Burlington, VT 05405-0105, USA
Address for correspondence: vgouli@uvm.edu

Delta endotoxins are related to homologous polypeptides incoming in structure of parasporal bodies which are produced by numerous subspecies of *Bacillus thuringiensis* Berliner (Schnepf, et al. 1985). These types of toxins attract attention of researchers as very effective tools for controlling noxious insect pests. Toxins have an influence on cell membranes of mid-intestine epithelium of susceptible insects. Contact of toxin with cell membranes has led to formation of the ion-specific pores destroying ion transport through membrane (Tran et al. 2001). At the same time, toxins provoke the peroxidal oxidation of lipids in cell membranes and act as a disconnector of phosphorylation and respiration processes (Kamenek, Shternshis, 1985). Condensation of mitochondria matrix, destruction of nucleoplasm and insect cell lysis is the most typical cytopathological consequences under influence of *Bt* endotoxins. In the last few years, new properties of endotoxins connected with antibacterial and antifungal activities were established. In particularly, delta-endotoxins take action on phytopathogenic fungi from different genera including *Fusarium*, *Bipolaris*, *Phytophthora*, *Alternaria*, *Rhizoctonia* and some others having significant importance for agriculture (Tulpanova, 2003). United structure of eukaryotic cells, especially arthropods and fungi gives the possibility to suppose the similar mechanism of *Bt* endotoxin action on both insect and fungal cells. But, at the present time, the mechanism of antifungal action of endotoxins is scantily explored. The information presented is devoted the activity of delta-endotoxin of *Bt subsp. thuringiensis* towards some plant pathogenic fungi. Uncoupling of oxidizing phosphorylation and respiration in the target fungi may be the possible mechanism of anti-fungal activity of endotoxins. The increase of respiration activity of the target fungi, similar to that of the phytopathogenic bacteria, was established. The dependency of the microorganism sensitivity on the endotoxin concentration and contact time with it was established. The phytoprotective activity of the endotoxin towards phytophthora-affected plants was shown using tomato and potato in storage environment.

Poster / Microbial Control, Monday 16:00 **MC18**

Role of the GntP protein in germination and outgrowth of *Bacillus thuringiensis*

Li-Fang Ruan, Guoqiang Liu, Yiming Wu, Jinshui Zhen, Dong-Hai Peng, Ming Sun
State Key Laboratory of Agricultural Microbiology, College of Life Science and Technology, Huazhong Agricultural University, Wuhan, 430070, China.
Addresses for correspondence: ruanlifang@mail.hzau.edu.cn; m98sun@mail.hzau.edu.cn.

Bacillus thuringiensis(*B.thuringiensis*), is a insect pathogen and is extensively used in biological pest control, has a dormant stage in its life cycle known as the endospore. The germination of endospore in haemocoel of insect is very important to the virulence of *B.thuringiensis*. The GntP(gluconate permease) is responsible for the import of gluconate or fructuronic acid, was identified as channel protein in *B.subtilis* and *E.coli* previously. In this study, a gntP deletion mutant of *B.thuringiensis* YBT1518 was obtained by randomly insertion of mini-Tn10 carried on plasmid pIC333. This mutant showed germination defects to nutrient germinants, like L-alanin, AGFK, L-valine, L-phenylalanine and 50mmol/L L-leucine except inosine. The complementation experiment confirmed that the gntP have relationship with germination defect phenotypes of YBT1518(gntP-).The GntP was localized to the spore outer membrane by transmembrane motif prediction and the germination assay of decoating YBT1518(gntP-). However, the GntP protein did not show the same function in model of Bacillus, *Bacillus subtilis*, by a knock-out experiment. The GntP protein was proposed as a channel protein responsible for the import of nutrient germinants.

Poster / Microbial Control, Monday 16:00 **MC19**

An 100 generations population *Plutella xylostella* susceptibility to *Bacillus thuringiensis*

Sérgio Antonio De Bortoli[1]; Ricardo Antonio Polanczyk[1]; Alessandra Marieli Vacari[1]; Caroline Placidi De Bortoli[1]
Plant Protection Department, Universidade Estadual Paulista. Via de Acesso Prof. Paulo Donato Castellane, s/n. 14884-900. Jaboticabal, Sao Paulo, Brazil.
Address for correspondence: rapolanc@fcav.unesp.br

Plutella xylostella (L.) is one the most important pests of crucifers worldwide and *Bacillus thuringiensis* Berliner based-biopesticides are used as alternative to chemicals that may have undesirables effects on environmental. However, reports of rapid resistance evolution of *P. xylostella* to *B. thuringiensis* based biopesticides were reported in some countries. A Brazilian population of *P. xylostella* with 100 generations was assayed to *B. thuringiensis* isolates susceptibility. Biossays were performed with seven *B. thuringiensis* isolates cultivated in nutrient agar and incubated for 5 days at 30^{o}C. Twenty six *B. thuringiensis* 0.5mL suspensions ranged from 9 x 10 to 3 x 10^{8} spores.mL^{-1} of each isolate were sprayed on 3 cm kale foliar dishes. Mortality was evaluated since 24 hours after treatment applications. Probit analyses were performed to estimate LC_{50}. Mortality was 100% to all isolates and data were adequate enough to estimate LC_{50} only to isolate T08.024 with an estimated value of 2 x 10^{6} spores.mL^{-1}. This population shows a very low rate of resistance evolution because after 100 generation at laboratory, the mortality was high to all tested isolates.

Poster / Microbial Control, Monday 16:00 **MC20**

Three new strains of *Bacillus thuringiensis* as potential biocontrol agent against pest weevils in the Republic of Moldova

Natalia V. Munteanu, Nadejda V. Malevanciuc, Anna I. Moldovan, Ion C. Toderas
Centre of General and Molecular Biology, Institute of Zoology, Moldova Academy of Science, 2028, Chisinau, Republic of Moldova.
Address for correspondence: natalia_v_munteanu@yahoo.com

Bacillus thuringiensis (*Bt*) is a bacterium commonly known as an important biocontrol agent. It is widely used as bioinsecticide for the control of many agricultural insect pests, and it constitutes the basis of over 90% of commercially available biopesticides. In this study, in order to detect and identify the most toxic *Bacillus thuringiensis* strains against pest weevil, we isolated *B. thuringiensis* strain Po4 from *Phyllobius oblongus* L., Ta16 strain from *Tatianaerhynchites aequatus* L. and Np1 strain from *Neocoenorhinidius pauxillus* Germ., the most economically

important insect pests of fruit trees in the Republic of Moldova and Europe. Control measures for this pest in Moldova are few and provide only limited population suppression. *Bt* strains were isolated from different weevil specimens collected from a range of host plants from spring 2008 through summer on the territory of the Republic of Moldova. Based on morphological, biochemical, and molecular techniques isolated strains were characterized as *Bacillus thuringiensis* subsp. *kurstaki*. The microscopy techniques indicated that Po4, Ta16 and Np1 isolates have crystals with bipyramidal shapes. The Polymerase Chain Reactions (PCRs) revealed the presence of the cry1 genes. The presence of Cry1 proteins in all isolates was confirmed via SDS-PAGE, at approximately 130 kDa. The bioassays were conducted with distinct weevil insects, using spore-crystal mixtures; all of the strains were able to kill adults of *Sitona* spp. and *Phyllobius* spp. The results show that the *B. thuringiensis* Po4, Ta16 and Np1 isolates may prove valuable as microbial control agent against curculionidae pests.

Poster / Microbial Control, Monday 16:00 **MC21**

A high-throughput strategy for identification of new toxin genes from *Bacillus thuringiensis* based on cosmically sequencing and computational pipeline

Donghai Peng†, Weixing Ye†, Lei Zhu, Yingying Liu, Ce Geng, Han Wu, Jinshui Zheng, Lifang Ruan, and Ming Sun*
State Key Laboratory of Agricultural Microbiology, College of Life Science and Technology, Huazhong Agricultural University, Wuhan, 430070, China.
† These authors contributed equally to this work.
Addresses for correspondence: donghaipeng@mail.hzau.edu.cn; m98sun@mail.hzau.edu.cn.

Discovery new insecticidal crystal proteins were considered as one of the major approaches to overcome the potential insect resistance to *Bacillus thuringiensis*. We developed a high-throughput strategy for identification new toxin genes from *B. thuringiensis* based on cosmically sequencing and a computational pipeline. Three rules (Percent Identity and Alignment Length rule, Hidden Markov Model rule, and Support Vector Machine rule) were constructed to recognize toxin protein. They achieved fine prediction with accuracy over 98% and Matthew's Correlation Coefficient (MCC) over 0.92. Then, a computational pipeline was developed based on these three rules and two databases (*B. thuringiensis* toxin database and background database). When compared to traditional annotation methods by using the reported *B. thuringiensis* whole genome sequences, the constructed pipeline showed much more powerful in precise and efficiency to annotate toxin genes. Meanwhile, the total plasmids from 21 *B. thuringiensis* strains were mixed together and sequenced by Illumina GA2 sequencer. Then, 364 positive toxin hits with different coverage were identified by constructed pipeline from this multiple *B. thuringiensis* admixture plasmids sequencing data. Fifty sequences were then selected due to their higher coverage and low identity to known toxin sequences for cloning and sequencing. Finally, 36 new holotpye toxin genes were identified (including 19 rank I, 15 rank II, and 2 rank III genes). We named this pipeline as BtToxin-Scanner and implemented it as a freely accessible web server at: http://122.205.95.26/BtToxin_scanner/. The admixture plasmid sequencing strategy combined with BtToxin-scanner would greatly accelerate the exploration of novel *B. thuringiensis* toxin proteins.

Poster / Microbial Control, Monday 16:00 **MC22**

Effect of *Bacillus thuringiensis* on the reproductive capacity and survival of the predator *Orius insidiosus*

R.M. Goulart, A.M. Vacari & S.A. De Bortoli
FCAV/Unesp – Dep. Fitossanidade – Laboratório de Biologia e Criação de Insetos (LBCI), Via de Acesso Prof. Paulo Donato Castellane s/n 14884-900 Jaboticabal, SP, Brazil.
Address for correspondence: bortoli@fcav.unesp.br

This study evaluated the action of *Bacillus thuringiensis* Berliner on the biological characteristics of the predator *Orius insidiosus* (Say) with the aim of studying the action of these bacteria on the physiological activity of the predator and its possible effect when the plant is sprayed with an entomopathogenic agent. The first group (eighty nymphs) of predators were fed with *Plutella xylostella* (L.) eggs immersed in water (control) and the second with *P. xylostella* eggs dipped in a suspension of *B. thuringiensis*. We used a concentration that could control the growth of *P. xylostella* on cabbage (1.4 g/ L), as recommended by the manufacturer of the commercial product Agree® (*B. thuringiensis* var. *aizawai* and *B. thuringiensis* var. *kurstaki*). The predators were offered 30 eggs (up to 24 hours old) that were glued on a light blue card (0.4 × 2.0 cm) everyday. After 24 hours, these cards were replaced with new cards in order to obtain the daily consumption. We evaluated the consumption and nymphal period. Twenty couples were individualized in petri dishes and each couple formed a replication. We evaluated the consumption, number of eggs per female, and egg viability. The characteristic second instar nymphal period and life-span of *O. insidiosus* were affected by the consumption of eggs immersed in Agree®. *O. insidiosus* females who consumed *P. xylostella* eggs that were immersed in the product Agree® had lower number of offsprings, thereby resulting in a lower population growth rate.

Poster / Microbial Control, Monday 16:00 **MC23**

Effect of *Bacillus thuringiensis* on the parasitism capacity of *Trichogramma pretiosum*

Roberto M. Goulart; Alessandra M. Vacari; Sergio A. De Bortoli
FCAV/Unesp – Dep. Fitossanidade – Laboratório de Biologia e Criação de Insetos (LBCI), Via de acesso Prof. Paulo Donato Castellane s/n 14884-900 Jaboticabal, SP, Brazil.
Addresses for correspondence: bortoli@fcav.unesp.br

The objective of our study was to evaluate the influence of product formulations of *Bacillus thuringiensis* Berliner (Agree®) on the biological characteristics by *Trichogramma pretiosum* Riley in the laboratory. We observed 40 replications of the following treatments: (1) *T. pretiosum* reared on diamondback moth (*Plutella xylostella* L.*)* by generating a parasitizing *P. xylostella* with immersion in a suspension of *B. thuringiensis*, (2) *T. pretiosum* reared on *P. xylostella* eggs parasitized by 2 generations of *P. xylostella* with immersion in a suspension of *B. thuringiensis*, (3) *T. pretiosum* reared on *P. xylostella* by generating a parasitizing *P. xylostella* with immersion in water - control, and (4) *T. pretiosum* reared on *P. xylostella* eggs parasitized by 2 generations of *P. xylostella* with immersion in water - control. The eggs were offered daily throughout parasitoids life cycle. The concentration of the suspension was maintained according to the manufacturer's recommendations for the commercial product Agree® (*B. thuringiensis* var. *aizawai* + *kurstaki*) in order to control the infestation of cabbage by the diamondback moth (1.4 g/L). We evaluated the following biological characteristics: daily parasitism, cumulative parasitism and percentage of emergence. The treatments showed similar values: the first generation control (52.82 eggs), the second generation control (48.93 eggs), and the second generation *B. thuringiensis* (50.78 eggs). In conclusion, the commercial product Agree® has no negative effects that could prevent its use with the parasitoid *T. pretiosum* for the integrated management of *P. xylostella* infesting crucifers.

Poster / Microbial Control, Monday 16:00 **MC24**

Interaction between *Bacillus thuringiensis* and *Telenomus remus*

João R. D. C. C. Alencar[1]; Ricardo A. Polanczyk[1]; Marina A. Viana[2]; Sergio A. De Bortoli[2]; Thiago T. Agostini[1]; Alessandra M. Vacari[2]; Jaqueline M. Maeda[2]
[1]Laboratory of Arthropod Pests Microbial Control, Universidade Estadual Paulista. Access route Paulo Donatto Castellane, Jaboticabal, SP 14884-900 Brazil. [2]Laboratory of Rearing and Biology of Insects, Universidade Estadual Paulista.Access route Paulo Donatto Castellane, Jaboticabal, SP 14884-900 Brazil
Address for correspondence: rapolanc@fcav.unesp.br

The fall armyworm, *Spodoptera frugiperda*, is on the the most important pest of maize in Latin America. The bacterium *Bacillus thuringiensis* (Bt) and the egg parasitoid *Telenomus remus* are biological control agents used in *S. frugiperda* IPM systems, but there is a lack of studies on interaction between these natural enemies. The aim of this study was to evaluate the interaction between *T. remus* and *B. thuringiensis* using eggs of *S.frugiperda* as host. A *B. thuringiensis* based biopesticide named Dipel was used in two treatments (8 replicates) at dose recommended by the manufacturer plus spreader-sticker (Tween ®80) 0.05%. In the first treatment, egg masses was sprayed in a Potter tower and in the second treatment food (honey) was added to the product in a 1:1 ratio. The parasitoid was deprived of food for 24 hours before bioassay. The control was conducted similarly but using deionized autoclaved water. The evaluated parameters were parasitism, sex ratio, viability of parasitism and data was submitted to the Tukey test (α =0.05). There are no differences between treatments indicating that both biological control agents can be used together in *S. frugiperda* IPM systems without negative interference between the egg parasitoid and the entompathogenic bacterium. However, it is necessary to conduct further studies involving other aspects such as longevity of the progeny, parasitism index and preference for the substrate.

Poster / Microbial Control, Monday 16:00 **MC25**

Interaction between Dipel® and *Orius insidiosus*

Jaqueline M. Maeda[1]; Alessandra M. Vacari[1]; Marina A. Viana[1]; João R. D. C. C. Alencar[2]; Caio Y. M. Yamauchi[1]; Sergio A. De Bortoli[1]
[1]Laboratory of Rearing and Biology of Insects, Universidade Estadual Paulista. Access route Paulo Donatto Castellane, Jaboticabal, SP 14884-900 Brazil. [2]Laboratory of Arthropod Pests Microbial Control, Universidade Estadual Paulista. Access route Paulo Donatto Castellane, Jaboticabal, SP 14884-900 Brazil.
Address for correspondence: amvacari@gmail.com

The generalist predator *Orius insidiosus* (Say) is able to prey several species of small arthropods such as thrips, mites, aphids and also lepidopteran eggs during its life cycle. Due to its feeding habit and their distribution in southern Canada, throughout the United States, West Indies, Brazil and Argentina this predator has become an important biological control agent. The aim of the research was to evaluate the mortality of *O. insidiosus* after spraying topical in the predator and crucifer leaves (*Brassica oleracea* var. *acephala*) of Dipel® to control diamondback moth. The experiment was conducted in a room at 25 ± 1º C, relative humidity of 70 ± 10% and photophase of 12 hours. The Dipel® was dissolved according to the manufacturer's recommendations in the concentrations: $1x10^{10}$, $1x10^{9}$ and $1x10^{6}$ spores.ml^{-1}. Were sprayed 10 replicates of each Dipel® concentrations and compared with the control with 0.05% Tween®80 (spreader-sticker). For the control were used deionized sterile water added of spreader-sticker. After spraying we added 5 larvae of *P. xylostella* and after 24 hours, was added to the predator. To evaluate the topical effect, adults of the predator were sprayed and placed in the same concentrations in leaves of crucifers unsprayed containing 5 larvae of *P. xylostella* and evaluated by 7 days. The results showed no significant differences between the spraying mode and the various concentrations of Dipel®. Different concentrations of Dipel applied leaf and predator showed no significant. Therefore, the two control modes can be used together to improve the control of diamondback moth.

Poster / Microbial Control, Monday 16:00 **MC26**

Charactrization of Iranian isolates of entomopathogenic nematodes for biocontrol of leopard moth, *Zeuzera pyrina* L. (Lep.: Cossidae)

Javad Karimi[1]; Mohammadreza Rezapanah[2];Mahbobeh Ashtari[3]; Reyhaneh Darsouei[1]; Raquel Campos-Herrera[4,5] ; Larry Duncan[4]
[1]Department of Plant Protection, Faculty of Agriculturey, Ferdowsi University of Mashhad, Mashhad, Iran, [2] Biological Control Dept., Iranian Research Institute of Palnt Protection, Tehran, Iran, [3] I.A. University of Arak, Iran, [4] University of Florida, IFAS, Citrus Research and Education Center, Lake Alfred, FL, 33850 USA, [5] Instituto de Ciencias Agrarias, CSIC, Madrid, 28006, Spain
Address for correspondence: jkb@um.ac.ir

The leopard moth, *Zeuzera pyrini* (Lep. Cossidae) is a destructive wood borer found throughout Iran. It poses unique management challenges because its immature stages live in cryptic, often inaccessible, habitats within host trees. Safety and environmental concerns associated with chemical control of this pest caused us to investigate the potential of native and non-native entomopathogenic nematodes (EPNs) to manage this caterpillar. Four strains of nematodes were isolated from soil in walnut orchards infested by *Z. pyrini* near Arak, Iran. The nematode isolates were identified from ITS and 28S rDNA (D2/D3) as *Heterorhabditis bacteriophora* (Arak1), *Steinernema carpocapsae* (Arak2) and *S.feltiae* (Arak3), respectively. The fourth isolate (Arak4) was identified as the free living rhabditid *Oscheius tipulae* which is a new genus and species record for Iran. The highest mortality of *Z. pyrini* larvae in laboratory and field experiments was caused by *S. carpocapsae*, but *H. bacteriophora* was also able to infect and kill this insect. The average insect mortality caused by *S. carpocapsae* in field trials was 63% and was increased to 90% by covering the insect galleries with plastic after EPNs were introduced. Both EPN species appear to be more effective against *Z. pyrini*, which occur in moist heartwood galleries, than against other insect species that reside in drier wood galleries.

Poster / Microbial Control, Monday 16:00 **MC27**

Cloning, characterization and expression of an insecticidal crystal protein gene from *Bacillus thuringiensis* isolates of Andaman and Nicobar Islands

H.M.Mahadeva Swamy[1], R.Asokan[1], D.K.Arora[2]
[1]Division of Biotechnology, Indian Institute of Horticultural Research (IIHR), Hessarghatta lake post, Bangalore 560089, Karnataka, India
[2]National Bureau of Agriculturally Important Micro organisms (NBAIM), Mau Nath Bhajan, 275101, Uttar Pradesh, India

Biocontrol of pests via *Bacillus thuringiensis* (Bt) δ-endotoxins represents the most successful use of a biological control agent to date. The most notable characteristic of *Bacillus thuringiensis* is its ability to produce insecticidal proteins. More than 300 different proteins have been described with specific activity against insect species. The six isolates of *Bacillus thuringiensis* from Andaman and Nicobar Islands which were previously characterized by PCR analysis for the presence of Coleopteran active *cry* genes were used for *Cry1I* full length gene amplification. A 2.16-kb DNA fragment of *Cry1I* gene was PCR amplified, cloned in expression vector pQE 80 L, and then used for transformation of *E. coli M15* cells. The optimum expression was obtained with 1 mM IPTG at 37^{0}C for 3 h. The sequence of the cloned crystal protein gene showed almost complete homology with a *Cry1I* toxin gene from *Bacillus thuringiensis* var. *kurstaki*, with scattered mutations in the toxic region. The deduced sequence of the protein has homologies of 91.0% with *Cry1I* and *Cry1Ia*, and 98.0% with *Cry1Ib*.Cloning

of this gene may help to overcome the increasing resistance of pests to currently used insecticides. Based on the results obtained, the PCR method may be a valuable and reliable tool for specific detection and identification of *cry1I* genes.

Poster / Microbial Control, Monday 16:00 **MC28**

Effect of *Bacillus thuringiensis* on the reproductive characteristics of *Podisus nigrispinus* fed on *Plutella xylostella*

Alessandra M. Vacari; Vanessa F. P. Carvalho; Gustavo O. Magalhães; Marina A. Viana; Sergio A. De Bortoli; Ricardo A. Polanczyk
FCAV/Unesp – Dep. Fitossanidade – Laboratório de Biologia e Criação de Insetos (LBCI), Via de acesso Prof. Paulo Donato Castellane s/n 14884-900 Jaboticabal, SP, Brazil.
Address for correspondence: amvacari@gmail.com

Bacillus thuringiensis Berliner toxins can affect non-target insects when they are consumed by predators of the last trophic level through the prey/host feeding on plants directly or when consuming it in the form of a suspension. Furthermore, this toxin moves up the trophic levels. The aim of this study was to evaluate the influence of *B. thuringiensis* on the reproductive characteristics of the predator *Podisus nigrispinus* (Dallas). The experiments were performed on Laboratory of Biology and Insect Rearing that were kept in a room at 25 ± 1°C, relative humidity 70 ± 10%, and photophase of 12 hours. The first group (sixty nymphs) was fed only healthy larvae of *Plutella xylostella* (L.) (control); the second was fed *P. xylostella* larvae that consumed kale leaves sprayed with a suspension of *B. thuringiensis* var. *kurstaki* HD1 at a concentration of 1.5 $\times 10^7$ spores/mL (infected larvae); and the third was fed healthy *P. xylostella* larvae, but the water that was placed in a tube (dental anesthetic) was replaced with a suspension of *B. thuringiensis* (suspension). The key reproductive characteristics of *P. nigrispinus* females were identical in all the groups. For the control, suspension, and infected larvae, fecundity was 321.9, 420.2, and 415.2 eggs per female; egg fertility was 82.0%, 63.7%, and 64.4%; and female longevity was 46.2, 49.4, and 58.3 days, respectively. These results show that consumption of either infected larvae or a suspension of the bacteria in water is not harmful to predators

Poster Papers Monday, 16:30-18:30

Diseases of Beneficial Invertebrates

Poster / DBI, Monday 16:00 **DBI-1 STU**

White Spot Disease mimics: hunt for the elusive B virus in European shore crabs (*Carcinus maenas*) and discovery of a novel herpes-like virus *en route*

Kelly S. Bateman and Grant D. Stentiford
European Union Reference Laboratory for Crustacean Diseases, Centre for Environment, Fisheries and Aquaculture Science (Cefas), Barrack Road, The Nothe, Weymouth, Dorset, DT4 8UB, UK.
Address for correspondence: kelly.bateman@cefas.co.uk

B Virus was discovered in 1974 (Bazin *et al.)* infecting *Carcinus maenas* from the French coastline. Whilst the taxonomic position of B virus has never been defined using a molecular phylogenetic approach, due to its morphological and pathological similarity to White Spot Syndrome Virus (WSSV), it has been tentatively classified within the *Nimaviridae* by the International Committee on Taxonomy of Viruses (Vlak *et al*., 2005). Since WSSV has recently been listed in EC Directive 2006/88, the historic presence within European waters of a pathogen that mimics the pathology of WSSV is important. We attempted to rediscover B virus to enable a phylogenetic comparison with WSSV, sampling juvenile *C. maenas* from several sites in the English Channel (type location for B virus) and various sites around the UK coastline. Tissues were processed for histology and electron microscopy to investigate the presence of pathogens. Using this approach, we identified pathology very similar to that described for B virus (and WSSV) in crabs from the English Channel. Haemocytes and connectives tissue cells displayed hypertrophied nuclei with marginalized chromatin and eosinophillic inclusion bodies. However, when tissues were processed for electron microscopy, infected cells did not contain virions conducive with B virus but rather those of a novel herpes-like virus (HLV). In this study, we describe the pathology and ultrastructure of HLV infection in *C. maenas* and highlight this novel virus as a further pathological mimic to B virus/WSSV in crabs. Further phylogenetic work is underway to characterise HLV. The search for B virus continues.

Poster / DBI, Monday 16:00 **DBI-2**

Histological survey of European shore crab *Carcinus maenas* from UK waters

Kelly S. Bateman, Ruth J. Hicks and Grant D. Stentiford
European Union Reference Laboratory for Crustacean Diseases, Centre for Environment, Fisheries and Aquaculture Science (Cefas), Barrack Road, The Nothe, Weymouth, Dorset, DT4 8UB, UK.
Address for correspondence: kelly.bateman@cefas.co.uk

The European shore crab *Carcinus maenas* is ubiquitous around the coastline of the UK and is known to be susceptible to a wide range of parasites and pathogens. Since this species is likely to be utilised by European Member States wishing to define their status for the listed pathogen white spot syndrome virus (WSSV) as stipulated in EC Directive 2006/88, we carried out a survey of *C. maenas* collected from numerous sampling points around the UK coastline between July and October 2010. Samples were taken for histology, electron microscopy and molecular diagnostics. We identified a wide range of viral, bacterial, protistan and metazoan pathogens and found that the apparent prevalence and pathogen profile differed considerably between sites. Notably, crabs obtained from one of the sampling sites (West Mersea, Essex) displayed up to 50% apparent prevalence of *Hematodinium perezi*, the type species of the genus, previously considered to be a low prevalence pathogen (<5%) in this species. The discovery of high prevalence at this site (near to the English Channel type location) offers significant potential for a full genetic characterisation of the type species and comparison to more recently described *Hematodinium*-like dinoflagellates from marine decapods.

Poster / DBI, Monday 16:00 **DBI-3**

PaV1 detection by the Caribbean spiny lobster and its effect on population spatial structure

Josh Anderson and Donald C. Behringer
University of Florida, School of Forest Resources and Conservation & Emerging Pathogens Institute, Gainesville, FL 32653 USA
Address for correspondence: andersonj@ufl.edu

PaV1 (*Panulirus argus* Virus 1) is a lethal contact-transmitted pathogen that infects the Caribbean spiny lobster *Panulirus argus* Latrielle. Juvenile lobsters are highly susceptible to infection, which causes tissue degradation, lethargy, and mortality. However, healthy lobsters have the ability to reduce infection risk by avoiding shelters inhabited by infected lobsters. Based on the role of olfaction in many aspects of lobster ecology such as conspecific attraction and mate searching, we hypothesized that it was the most likely mechanism by which lobsters detect PaV1. We used a series of y-maze experiments to test this hypothesis and determine the source of the olfactory cue. Shelter avoidance behavior also has the potential to alter the population spatial structure, and based on the type of cue, could be affected by local hydrodynamics. To investigate this we manipulated shelter and disease cues for wild populations in both high and low flow environments. Results

showed that disease avoidance is driven by olfaction via urine release, and moreover, the olfactory cue alone was equivalent in effectiveness to having a diseased lobster present and visible. When given a choice between sheltering with diseased or healthy conspecifics, lobsters rarely sheltered with diseased individuals. In shelter-limited environments, as can occur with sponge die-offs, the unavailability of shelter due to disease avoidance could result in increased exposure to predation.

Poster / DBI, Monday 16:00 **DBI-4**

Prevalence of viral sequences in honey bees from the U-Mass apiary

Ajanta De[1]; John P. Burand[1,2]
Departments of [1]Microbiology, and [2] Plant, Soil and Insect Sciences, University of Massachusetts – Amherst, Amherst, MA 01003 USA
Address for correspondence: jburand@microbio.umass.edu

Honey bees (*Apis mellifera*), from hives maintained in an apiary on the University of Massachusetts - Amherst campus were analyzed over the past two years for the presence of viral pathogens using reverse transcription-PCR (RT-PCR). Either pools or samples of individual bees from these hives were examined for 3 plus strand RNA honey bee viruses including: black queen cell virus (BQCV), deformed wing virus (DWV), sacbrood virus (SBV). While the level of BQCV in these bees remained high throughout this period ranging from 70 to over 95%, while levels of SBV and DWV varied considerably with SBV being found between 25 to 90% and DWV being present at levels ranging from 30 to 100%. Surprisingly, in our analysis of bees collected this spring while the infection rate of BQCV remained high at 90%, DWV in these bees dropped below our level of detection. It is interesting to find that while the prevalence of some viruses like DWV and SBV fluctuates dramatically and may actually disappear from the hive while in the same bees the level of BQCV remains consistently high. We are continuing to examine bees from hives in this apiary in an attempt to better understand what is responsible for the different patterns of viral prevalence we have observed to date.

Poster / DBI, Monday 16:00 **DBI-5**

Detection of Honey bee Virus Sequences in Native Bee Species from the Maine Blueberry Barrens

Crystal Cabral[1]; Anna Morkeski[2]; Anne Averill[2]; John P. Burand[1,2,3]
[1]Graduate Program in Molecular and Cellular Biology and the Departments of [2]Plant, Soil and Insect Sciences, [3]Microbiology, University of Massachusetts – Amherst, Amherst, MA 01003 USA.
Address for correspondence: jburand@microbio.umass.edu

Honey bees (*Apis mellifera*), bumble bees *(Bombus sp.)* and several native bee species were collected from several sites in the Maine blueberry barrens and analyzed for the presence of honey bee viruses using reverse transcription-PCR (RT-PCR). Either pools or samples of individual bees from these sites were examined for the presence of seven plus stranded RNA honey bee viruses including: Israel acute paralysis virus (IAPV), Kashmir bee virus (KBV), black queen cell virus (BQCV), deformed wing virus (DWV), sacbrood virus (SBV), acute bee paralysis virus (ABPV), and chronic bee paralysis virus (CBPV). None of the samples was found to contain KBV, IAPV, ABPV or CBPV. Analysis of individual bees from one site revealed that 94% of the 17 *Apis* samples were positive for DWV, SBV and BQCV sequences while 45% of *Bombus* were positive for all three of these viruses. Of the other bee species examined for the presence of viral sequences, 35% were positive for all three. While DWV was the most common virus found, ranging from 100% in *Apis* and other bee species to 80% in *Bombus* bees, BQCV was the least prevalent, being present in only 35% of the other bee species. These data suggest that these three honey bee viruses are shared among bee species in this site, however the source and the genetic relatedness of these viruses still needs to be determined.

Poster / DBI, Monday 16:00 **DBI-6 STU**

Identification of first toxins of *Paenibacillus larvae*

Anne Fünfhaus, Lena Poppinga, Elke Genersch
Institute for Bee Research, Friedrich-Engels-Str. 32, 16540 Hohen Neuendorf, Germany
Address for correspondence: elke.genersch@rz.hu-berlin.de

The Gram-positive bacterium *Paenibacillus larvae* is the causative agent of American Foulbrood, a notifiable epizootic of honey bee larvae. The disease process in individual larvae can be divided into a non-invasive and an invasive phase. The non-invasive phase at the beginning of infection is characterized by mass proliferation of *P. larvae* in the larval midgut lumen. During the invasive phase, *P. larvae* enters the haemocoel by breaching the intestinal epithelium of honey bee larvae accompanied or initiated by a visible rounding-up of some epithelial cells. Toxins and proteases are most likely involved in this process. Good toxin candidates are ADP-ribosylating AB-toxins, which are expressed by *P. larvae* and which are known to destroy the cytoskeleton and cell-cell contacts of the host. AB-toxins are comprised of a catalytic A-subunit and a B-subunit which induces translocation and targeting of the host cell. We recently identified three ADP-ribosylating AB-toxins, the *P. larvae* toxins Plx1, Plx2 and Plx3. For functional characterization these toxins were cloned in appropriate expression vectors. The recombinant plasmids were translated by using an *E. coli*-based *in vitro* expression system, or *in vivo* in *E. coli* BL21 C43 (DE3). SDS-gel- and Western blot analyses revealed a successful expression of the toxins. Purification of the proteins were performed by affinity-binding of the different tags to magnetic particles. Purified recombinant *P. larvae* toxins are now available for functional assays to verify toxin functions and to identify the cellular target molecules.

Poster / DBI, Monday 16:00 **DBI-7**

Preliminary molecular evidence for transcriptionally variant innate immune receptors in a model marine brachyuran decapod.

Chris Hauton[1]; Meggie Hudspith[1]; Richard Edwards[2]; Tim Elliott[3]
[1]School of Ocean and Earth Sciences, University of Southampton, National Oceanography Centre, European Way, Southampton, Hants, SO14 3ZH, UK; [2]School of Biological Sciences, University of Southampton, Highfield, Southampton, SO17 1BJ, UK; [3]Faculty of Medicine, University of Southampton, Somers Cancer Research, Building (MP824), Southampton General Hospital, Tremona Road, Southampton, SO16 6YD, UK.
Address for correspondence: ch10@noc.soton.ac.uk

Conventionally, the innate immune responses of invertebrates have been characterized as germ-line encoded systems which do not exhibit immune memory, adaptivity or specificity. However, within the last ~10 years phenomenological evidence has been presented to support concepts of specificity and memory within invertebrate taxa. Whilst the interpretation of some of these data has proved controversial, there have been very recent mechanistic reports of a diverse assemblage of pathogen receptor proteins, including immunoglobulin superfamily receptors (e.g. Dscam) from a limited number of invertebrate species. The sequences of some of these receptors demonstrate transcriptional rearrangement (exon-splicing) which could support theories of the evolution of immune specificity and memory within the invertebrate immune systems. If widespread, these receptors might provide a mechanism to support the logical design of novel therapeutics for the crustacean aquaculture industry. In this poster we review the

available data on DSCAM receptors in invertebrate phyla and present early results on the presence of a transcriptionally-variable DSCAM receptor in different, geographically remote, populations of the European shore crab *Carcinus maenas* (L.)

Poster / DBI, Monday 16:00 **DBI-8 STU**

Unravelling the function of secondary metabolites of *Paenibacillus larvae*

Garcia-Gonzalez, E[1]; Borriss, R[2]; Genersch, E[1]
[1]Länderinstitut für Bienenkunde, Molekulare Mikrobiologie und Bienenkrankenheiten, 16540 Hohen Neuendorf
[2]Humboldt Universität, Bakteriengenetik, Chaussestr. 117, 10115 Berlin
Address of correspondence: eva.garcia-gonzalez@cms.hu-berlin.de

American foulbrood (AFB) is considered the most contagious and destructive infectious disease in honeybees, caused by the Gram-positive, spore-forming bacterium *Paenibacillus larvae* (Genersch et al., 2006). Recently, comparative genome analysis revealed that *P. larvae* harbours giant gene clusters that code for polyketide synthases (PKS) and nonribosomal peptide synthetases (NRPS). These enzymatic complexes are responsible for the biosynthesis of natural products that are implicated in multiple functions such as antibiotic, immunosuppressive, cytostatic and toxic activity (Koumoutsi *et al*., 2004). We hypothesized that *P. larvae* requires these substances in order to successfully compete against bacterial competitors present in the larvae, i.e. that the substances produced by these NRPS serve as antibiotics. On the other hand some PKS-related compounds have been proven to be highly toxic and were associated with virulence. In *P. larvae* genome, we so far identified, extended, and assembled three putative NRPS/PKS clusters. Using bacterial growth inhibition assays we were able to demonstrate that *P. larvae* strains indeed inhibited the growth of different bacterial species suggesting that at least some of these NRPS/PKS clusters are functional and lead to non-ribosomal antibiotic production. A knock-out mutant for one of these clusters (NRPS-cluster II) was constructed and showed a dramatic decrease in bacterial inhibition activity confirming antibiotic activity of the substance produced by cluster II-NRPS. Further experiments, both in vitro and in vivo together with chemical characterization (HPLC-ESI-MS), will be performed to prove the role of these substances in pathogenesis.

Poster / DBI, Monday 16:00 **DBI-9**

Population genetics and pathogens of the Edible crab *Cancer pagurus* in the Irish Sea

Joseph E. Ironside[1], Andrew Rowley[2], Hayley Watson[1], Emma Wootton[2], Joanne Porter[3], Shelagh Malham[4].
[1]Institute of Biological, Environmental and Rural Sciences (IBERS), Edward Llwyd Building, Aberystwyth University, Aberystwyth, Ceredigion SY23 3DA, UK. [2]Department of Biosciences, College of Science, Swansea University, Singleton Park, Swansea, SA2 8PP, U.K. [3]School of Life Sciences, Heriot Watt University, John Muir Building, Gait 1, Riccarton Campus, Edinburgh [4]School Ocean Sciences, Bangor University, Menai Bridge, Anglesey, LL59 5AB, UK.
Address for correspondence: jei@aber.ac.uk

Edible crab (*Cancer pagurus*) is of high economic value to the UK and Ireland and is being exploited with increasing intensity due to the decline of fin-fisheries. The interdisciplinary EU project SUSFISH aims to provide information that will underpin sustainable development of Irish Sea shellfisheries for the next 50-100 years. To this end, we are studying the genetic structure of *C. pagurus,* its resilience to changes in its physical environment and its interactions with existing and emerging pathogens. Here, we focus upon preliminary studies of the effective population size and genetic structure of *C. pagurus* populations in the Irish Sea, together with baseline studies of diseases such as *Hematodinium* and shell disease. This data will be combined with oceanographic models and sociological studies to form a multifaceted picture of the current status and likely future trajectories of this important fishery.

Poster / DBI, Monday 16:00 **DBI-10 STU**

Potential siderophore in *Paenibacillus larvae*

Gillian L Hertlein; Eva Garcia-Gonzalez; Lena Poppinga; Anne Fünfhaus; Elke Genersch
Institute for Bee Research, Friedrich-Engels-Str. 32, 16540 Hohen Neuendorf, Germany.
Address for correspondence: elke.genersch@rz.hu-berlin.de

Honey bees are among the most important pollinators, playing a vital role in containing the richness of species among pollinating wild plants. Furthermore managed honey bees play an enormous economic role, as they are used for pollination of many crops and fruit.
Like any animal, bees face threats through pathogens (viruses, bacteria, fungi and parasites). An especially dreadful disease is the American Foulbrood, caused by the gram -positive spore forming bacteria *Paenibacillus larvae*. As suggested by its name, the fatal disease affects the larval stage: *P. larvae* proliferates in the midgut of bee larvae after ingestion of spores, lives like a commensal and until the whole cavity is filled. Then it breaches through the epithelium and thereby kills its host. The cadaver is decomposed to a ropy mass. As the entire vegetative stage proceeds inside the larvae, all micronutritions have to be stolen from the host – the most valuable among them being iron. Other pathogenic bacteria (e.g. *Bacillus anthracis*) established methods for extracting iron from the host proteins using siderophores. These are powerful and selective iron chelators, specifically secreted in response to iron deficiency. Comparative genome analysis revealed that *P. larvae* harbours a giant gene cluster, coding for nonribosomal peptide synthetases (NRPS), which might produces siderophores. *In vivo* and *in vitro* experiments to determine the role of the NRPS in pathogenesis will be carried out and first results will be shown.

Poster / DBI, Monday 16:00 **DBI-11**

A novel *Haplosporidium* sp. in shore crab (*Carcinus maenas*) and edible crab (*Cancer pagurus*): pathology, ultrastructure and phylogenetics

Stentiford, G.D.[1], Bateman, K.S.[1], Feist, S.W.[1], Stokes, N.A.[2], Carnegie, R.B.[2]
[1]European Union Reference Laboratory for Crustacean Diseases, Centre for Environment, Fisheries and Aquaculture Science (Cefas), Weymouth Laboratory, Weymouth, Dorset DT4 8UB, United Kingdom
[2]Virginia Institute of Marine Science, College of William & Mary, PO Box 1346, Gloucester Point, VA 23062, USA
Addresses for correspondence: grant.stentiford@cefas.co.uk

Previously, we described the pathology and ultrastructure of a novel haplosporidian-like parasite infecting the common shore crab (*Carcinus maenas*) from the European shoreline. Subsequently, surveys of juvenile life stages of European edible crabs (*Cancer pagurus*) residing in similar littoral habitats have revealed another haplosporidian-like parasite, albeit with a different pathological outcome to the *C. maenas* parasite, in up to 70% of juvenile life stages. The parasite is apparently absent from adults of the same species sampled immediately offshore from collection sites. Histology demonstrated hypertrophy of the antennal gland and bladder (excretory organs) of infected *C. pagurus*. Epithelial cells of the affected organs contained large numbers of apparently uninucleate and multinucleate life stages. Ultrastructural observations using transmission electron

microscopy revealed the haplosporidian-like parasite progressing from uninucleate life stages to multinucleate plasmodia and eventually to large inclusions containing once again, uninucleate life stages. Despite analysis of several hundred specimens, spore stages were not observed in any infected crab. All of these stages were apparently liberated from the apical portion of infected host cells into the lumen, presumably for excretion via the urine. For phylogenetic analysis, genomic DNA was extracted from the gill or hepatopancreas of two infected *C. pagurus* and also from two examples of haplosporidian-infected *C. maenas* collected in previous studies. Small subunit ribosomal DNA of the pathogen was amplified by PCR before cloning and sequencing. All four crabs yielded an identical 1736bp parasite sequence. BLAST analysis against the NCBI GenBank database identified the sequence as most similar to the protistan pathogen group comprising the haplosporidians. Parsimony analysis placed the crab pathogen within the genus *Haplosporidium*, sister to the gastropod and bivalve molluscan parasites *H. montforti*, *H. pickfordi* and *H. lusitanicum*. Ongoing work includes in situ hybridization of parasite stages and sequencing of the ITS genomic regions. The presence of an apparently asporous haplosporidian parasite infecting decapod crustaceans from the European shoreline, and with close affinity to previously described spore forming haplosporidians infecting molluscs is intriguing and may shed considerable light on the ecology of this enigmatic parasite group.

Poster / DBI, Monday 16:00 **DBI-12 STU**

Functional analysis of the S-layer protein of *Paenibacillus larvae*

Lena Poppinga[1]; Anne Fünfhaus[1], Eva Garcia-Gonzalez[1], Bettina Janesch[2], Christina Schäffer[2], Elke Genersch[1]
[1]Institute for Bee Research, Molecular Microbiology and Bee Pathology, Hohen Neuendorf, Germany
[2]University of Natural Resources and Life Sciences, Vienna, Austria
Address for correspondence: elke.genersch@rz.hu-berlin.de

Honey bees are the most important pollinators in agriculture providing about 90% of the commercial pollination of those crops and fruit which depend on insect pollination. Honey bees are attacked by numerous pathogens and parasites causing considerable economical losses to apiculture and agriculture. The notifiable honey bee epizootic American Foulbrood (AFB) is a bacterial disease solely affecting the brood of the European honeybee (*Apis mellifera*). The causative agent of AFB is the Gram-positive bacterium *Paenibacillus larvae*, which forms extremely resilient spores serving as the transmission stage of the bacterium. In Europe, outbreaks of American Foulbrood are caused by the two differentially virulent *P. larvae* genotypes ERIC I and ERIC II. We applied comparative proteomics to unravel putative factors which will help to explain the observed virulence differences between the two genotypes. 2D-SDS-PAGE revealed the expression of an S-layer protein in ERIC II but not in ERIC I strains. Sequence analysis confirmed that the S-layer protein gene is non-functional in ERIC I due to an ERIC I-specific frameshift-mutation. Knockout-mutants were constructed to functionally analyze the S-layer protein. The S-layer-ko-mutant 04-309 S-layerΔ101 differed from the wildtype 04-309wt in colony morphology, adhesion capacity and behaviour in infected larvae.

Poster / DBI, Monday 16:00 **DBI-13**

The effects of eugregarinid protozoan, *Cephaloidophora pacifica*, on the Antarctic krill, *Euphausia superba*

Kunio T. Takahashi
National Institute of Polar Research, 10-3, Midori-cho, Tachikawa-shi, Tokyo 190-8518, Japan.
Address for correspondence: takahashi.kunio@nipr.ac.jp

The Antarctic krill, *Euphausia superba*, is a key species in the Antarctic food web because of its exceptionally high proportion in the biomass and its importance as a prey for animals at higher trophic levels. It frequently swarms and is the subject of significant commercial fishing. The eugregarinid protozoan, *Cephaloidophora pacifica* Avdeev (Order Eugregarinorida, Family Cephaloidophoridae) has previously been found in the digestive tract of *E. superba*. Gregarines are commonly found in the guts of many invertebrate groups, and are known as cosmopolitan. However, their impact on the host are known only in a few insects. We investigated the characteristics of eugregarine parasites on Antarctic krill in order to explore their possible impact on their host. Eugregarines in the diverticulum of the mid-gut gland appear to damage microvilli, which are involved in the uptake of digested nutrients and secretion of various enzymes. Their heavy infections in the mid-gut gland are pathogenic and significantly compromise host nutrition. Therefore, their parasites have the potential to physiologically harm the host causing reduced growth. We also found eugregarines in the host hind-gut, blocking the passage of food. Eugregarine infections may have a negative impact on digestion and absorption in the host digestive tract.

Poster / DBI, Monday 16:00 **DBI-14**

Electrical stunning: a suitable method for euthanising decapods crustaceans in research laboratories?

Ruth J. Hicks; Kelly S. Bateman; Grant D. Stentiford
European Union Reference Laboratory for Crustacean Diseases, Centre for Environment, Fisheries and Aquaculture Science (Cefas), Barrack Road, Weymouth, Dorset. DT4 8UB, United Kingdom
Address for correspondence: ruth.hicks@cefas.co.uk

Euthanasia of decapods crustaceans utilised for research purposes currently relies on a range of techniques that generally fall outwith national and international guidelines for animal welfare (e.g. chilling, chloroform exposure etc.). The Crustastun™, a device designed for the rapid euthanasia of decapods crustaceans, has recently been developed for use by the food industry. The device administers a lethal electric shock (110 volts, 2-5 amps for up to 10 seconds), killing the animal rapidly. Although designed principally for usage by the food industry, the practicality and relatively humane killing method of the machine (demonstrated by cessation of spontaneous activity in the central nervous system following stunning), coupled with its reasonable cost, make it a potentially useful tool for use in research laboratories. In order to investigate the effect of electrical stunning on the histological structure of crustacean tissues, we directly compared tissues and organs collected from edible crabs (*Cancer pagurus*), European lobsters (*Homarus gammarus*) and signal crayfish (*Pacifastacus leniusculus*), euthanised using either the Crustastun™, or via prior chilling on ice (a standard procedure utilised in laboratories for preparing crustaceans for dissection). Full histopathological and electron microscopical analyses were carried out on a range of tissues and organs (hepatopancreas, muscle, heart, gonad, nerve, gill) from crustaceans euthanized using the different approaches. Here we present the results of this study and provide an initial assessment on the potential suitability of electrical stunning for the euthanasia of decapods crustaceans in the research laboratory setting.

Poster / DBI, Monday 16:00 **DBI-15**

Activation of eastern oyster (*Crassostea virginica*) hemocytes following administration of β-glucans

Robert S. Anderson[1]; Gulnihal Ozbay[2]; David H. Kingsley[3]; Maureen A. Strauss[1]
[1]Chesapeake Biological Laboratory, University of Maryland Center for Environmental Science, Box 38, Solomons, MD 20688,

[2]Department of Agriculture and Natural Resources, Delaware State University, 1200 North DuPont Highway, Dover, DE 19901, [3]U S Department of Agriculture, Agricultural Research Service, Food Safety and Intervention Technologies, Delaware State University, W.W. Baker Building, 1200 North DuPont Highway, Dover, DE 19901
Address for correspondence: anderson@cbl.umces.edu

C. virginica hemocytes are important immune defense cells involved in antimicrobial activities and control of infectious diseases. Hemocytes are analogous to phagocytes of higher animals, such as macrophages that bear receptors for pathogen-associated molecular patterns, including β-glucans typical of yeasts, fungi, etc. β-glucans are high-molecular-weight glucose polymers sometimes used as immunostimulants in fish and crustacean aquaculture. In this study, a laboratory grade (zymosan A) or a commercially available immunostimulant form (MacroGard) of 1,3/1,6 β-glucan (200 µg/g wet weight) was injected into the adductor muscle hemolymph sinus of adult oysters. Morphological (total and differential hemocyte counts, and hemocyte aggregation) and functional (virus inactivation) signs of immune activation were examined at various times post injection. A rapid (24 h) elevation of total circulating hemocytes, persisting for as long as 10 days, was typically observed. Both β-glucans produced significantly increased percentages of circulating granulocytes, which are the most immunologically active hemocyte subtype. Hemocyte aggregation *in vitro* was also promoted by β-glucans; clumping is a typical consequence of cell activation. Oyster hemocytes are potential reservoirs for pathogenic human noroviruses. Hemocyte homogenates from β-glucan-treated or control oysters were incubated with murine norovirus (MNV-1); changes in viral titers were quantified via a plaque assay using a murine macrophage line (RAW 264.7). Antiviral activity of homogenates from β-glucan-treated oysters was dose-dependent and significantly greater than those of untreated oysters. Therefore, β-glucans may serve as immune modulating agents in *C. virginica*, producing hemocyte activation and possibly enhanced host resistance.

Microsporidia Workshop Presentation, Monday 21:00 **45**

Extreme dimorphism in a microsporidian infecting the musculature of marine crabs

Stentiford, G.D., Bateman, K.B., Feist, S.W., Chambers, E., Stone, D.M.
European Union Reference Laboratory for Crustacean Diseases, Centre for Environment, Fisheries and Aquaculture Science (Cefas), Weymouth, Dorset DT4 8UB, United Kingdom.
Address for correspondence: grant.stentiford@cefas.co.uk

The current taxonomy of the Phylum Microsporidia is being increasingly challenged by the use of nucleic acid-based approaches to phylogeny. The contradiction between morphology-based taxonomy and that based upon phylogenetics is problematic when attempting to describe novel taxa. A serendipitous discovery by our laboratory has provided a key example of this issue by demonstrating the potential for extreme dimorphism in a microsporidian parasite infecting a single cell type of a single species of marine crab (the common European shore crab *Carcinus maenas*). In this case, the microsporidian parasite appears to alternate between a primarily diplokaryotic lineage which culminates in unusual monokaryotic needle-like spores (*Nadelspora*-type), and a principally monokaryotic lineage that culminates in monokaryotic spores with pronounced surface projections (*Ameson*-type). Both lineages occur in direct contact with the cytoplasm of host muscle cells and can exist simultaneously in the same cell. Chance inclusions of the microsporidian parasites *Nadelspora canceri* (from the marine crab *Cancer magister*) and *Ameson michaelis* (from the marine crab *Callinectes sapidus*) in previously published phylogenetic assemblages based upon partial sequences of the SSU rRNA gene have demonstrated (though not discussed) a very close relationship between these two parasite genera, despite the fact that their described spore morphology and developmental cycle is very different, and in different hosts. Further analysis of the SSU rRNA gene in infected *C. maenas* appears to confirm genetic synonymy of the two spore types. The discovery reported here provides evidence that the morphologically divergent genera *Ameson* and *Nadelspora*, both previously described infecting the musculature of marine crabs, are potentially life cycle variants of the same taxon. Furthermore, they appear to form a clade with other morphologically diverse but phylogenetically and ecologically similar muscle-infecting microsporidians from marine crustacean hosts.

Virus Division Workshop Presentation, Monday 21:00 **46**

Virus taxonomy: Your responsibilities and that of the International Committee on Taxonomy of Viruses (ICTV)

Peter J. Krell[1]
[1]Department of Molecular and Cellular Biology, University of Guelph, Guelph ON N1G 2W1 Canada
Address for correspondence: pkrell@uoguelph.ca

Taxonomy is as exciting as watching paint dry, though much less appreciated than a striking Van Gough or Picasso that results. The International Committee on Nomenclature of Viruses (ICNV) in 1966 brought order to chaos by providing guidance and consistency to naming viruses. By 1975 ICNV became the International Committee on Taxonomy of Viruses (ICTV) recognizing that viruses form natural taxonomic groupings. Currently there are 2,285 virus species, 348 genera, 87 families (8 for insect DNA viruses), 19 subfamilies and 6 orders. New viruses, including insect viruses, continue to be discovered and all need a taxonomic home. ICTV reviews viral taxonomy reflecting the fluid nature of the taxonomic divisions but does not work in isolation. The virology community provides the guidance and suggestions (http://www.ictvonline.org) for the annual ICTV meetings (e.g. Sapporo Japan Sept 9-11, 2011). The invertebrate virus subcommittee chair (currently Peter Krell) answers to 10 Study Groups (e.g. one for *Baculoviridae*, one for *Tetraviridae*). New taxonomic proposal are usually coordinated through a Study Group while the Subcommittee Chair presents the proposal to the ICTV. Submissions range from the mundane of defining new species to more radical, like introducing a new Family (e.g. *Hytrosaviridae*) and altering the taxonomic hierarchy to better reflect the true character of viruses (e.g. four new genera for *Baculoviridae*). The advent of high throughput metagenomic virome sequencing and bioinformatics challenges the ICTV in its decision making, forcing the question; To what extent should a nucleic acid sequence from an environmental sample inform taxonomic decisions?

TUESDAY – 9 August

Symposium (Nematodes Division) Tuesday, 8:00-10:00
Sobey 260

Entomopathogenic nematodes as model systems for biological studies

Organizers: Glen Stevens and Ed Lewis

Symposium Paper, Tuesday 8:00 **47**

Parasites in the dirt: the case for entomopathogenic nematodes as models

Ed Lewis
Departments of Nematology and Entomology, University of California, Davis, CA 95616, USA
Address for correspondence: eelewis@ucdavis.edu

Model systems in general use familiar animals and plants with well-known life histories to ask questions about how the world works. Behavioral ecology has a rich tradition of using very well developed models to study topics ranging from imprinting geese to predation by lynxes. There are few models based on parasites (other than how they affect vertebrate hosts), and still fewer that occur in the soil. Entomopathogenic nematodes are well-known organisms, mainly due to their importance as biological control agents of agricultural pests, and can serve as models for the behavioral ecology of some parasites. Using two different theoretical frameworks that are used to describe entomopathogenic nematode behavior, we can develop and test several different hypotheses about how a parasite should make decisions. My first example will focus on how nematode foraging strategy can be used as a tool to make predictions about seemingly unrelated aspects of their biology. The second example will show how risk-sensitive foraging theory can explain parasite infection strategies.

Symposium Paper, Tuesday 8:25 **48**

EPNs as model systems for experiential learning in biology

Glen N. Stevens
School of Natural Sciences and Mathematics, Ferrum College, Ferrum, VA, 24088, United States
Address for correspondence: gstevens3@ferrum.edu

Entomopathogenic nematodes may be very suitable for inquiry-based labs in undergraduate courses such as Biology, Ecology, or Animal Behavior. The nature of the host-nematode-bacteria system allows for discussion and multi-week investigation of concepts such as symbiosis, mutualism, foraging behavior, statistical analysis, and comparison of closely-related species. Many of these concepts receive only limited focus in three-hour lab sections. The host-nematode-bacteria system also allows students to design their own research projects that answer questions they design once they become familiar with the basic biology of the system. This presentation will discuss the perceived benefits of multi-week, inquiry-based labs using EPNs that have been conducted in both Intro Biology and Intro Ecology courses in a small, liberal-arts college environment. It will use examples from the students' own presentations to illustrate concepts the students appear to have taken away from the experiment, and will solicit audience feedback regarding other techniques and approaches that could prove successful in this context.

Symposium Paper, Tuesday 8:50 **49**

Entomopathogenic nematodes as model predators in soil food webs

Robin J. Stuart[1]; Raquel Campos–Herrera[1,2]; Fahiem E. El–Borai[1,3]; Ekta Pathak[1]; Larry W. Duncan[1]

[1]Entomology and Nematology Department, University of Florida, IFAS, Citrus Research and Education Center,700 Experiment Station Road, Lake Alfred, FL 33850–2299, USA; [2]Instituto de Ciencias Agrarias, CSIC, Serrano 115 dpsp Madrid 28006, Spain; [3]Plant Protection Department, Faculty of Agriculture, Zagazig University, Zagazig, Egypt.
Address for correspondence: rstuart@ufl.edu

Entomopathogenic nematodes (EPNs) are important predators of insects in natural and managed ecosystems but their role in soil food webs and the various biotic and abiotic factors that influence their spatial and temporal dynamics remain obscure. In Florida, EPNs have been used as augmentative biological control agents of a major citrus pest, the root weevil, *Diaprepes abbreviatus* (L.). Root weevils tend to be a more serious problem in citrus groves planted on poorly-drained fine-textured flatwoods soils than in those on well-drained coarse sandy soils of the central ridge. EPN applications provide effective short-term control but EPN numbers in treated plots typically fall below pre-treatment or control-plot levels within weeks after application, and applications are often more effective in ridge soils than flatwoods soils. Experiments using caged sentinel weevil larvae indicate that endemic EPNs are more diverse and either more abundant or more effective predators in ridge groves than in flatwoods groves; and recent evidence indicates that differential susceptibility of EPN species to trapping and endoparasitic nematophagous fungi could contribute to differences in EPN communities among these habitats and, thereby, the ability of endemic EPNs to regulate weevil populations. Planting citrus trees in coarse sand mesocosms within flatwoods groves enhances the persistence of inoculated EPNs in mesocosms and could constitute an effective EPN conservation biological control strategy. Current studies of EPNs and their role as predators and prey in Florida citrus groves using quantitative real–time PCR (qPCR) are providing additional information on the functional dynamics of these soil food webs.

Contributed Papers Tuesday, 9:15-10:00

Nematodes 1

Contributed Paper, Tuesday 9:15 **50**

Host-parasite interactions between mermithid nematodes and mosquitoes

Randy Gaugler;[1] Manar Sanad[1,2;] M.M. Shamseldean[2]; Yi Wang[1]
[1]Center for Vector Biology, Rutgers University, New Brunswick, NJ USA
[2]Department of Zoology, Cairo University, Cairo, Egypt
Address for correspondence: gaugler@rci.rutgers.edu

The mermithid nematodes *Romanomermis iyengari* and *Strelkovimermis spiculatus* are biological control agents attacking a range of mosquito species. We compared various life cycle parameters of these parasites in laboratory exposures against larvae of *Culex pipiens pipiens*. Host search by preparasites (J2) was directed rather than random as indicated in previous studies. Preparasites of both species recognize and prefer to attack uninfected host larvae, a strategy resulting in greater female production. Infection reduces host heart rate and the reduction was correlated with increasing parasitic load. Post-parasite (J3) host emergence location differed sharply, with 93.2% of *R. iyengari* exiting from the anterior prothorax, whereas 100% of *S. spiculatus* emerged perianally. *R. iyengari* exhibits protandry with male post-parasites emerging before females, reflecting the intense male-male competition characteristic of this species; *S. spiculatus* males and females emerge from the host concurrently. Both mermithids displayed the same effect of parasite load on sex ratio, with male production increasing as load increases. As parasite load increased and host resources therefore were depleted more quickly, developmental time in the host decreased. Mating and oviposition occurs within a mating ball or cluster. Females in clusters show accelerated developmental time from post-parasite to the adult

stage and mate multiple times. The study of mermithid behavior and factors affecting their life cycle enhances their potential for mass rearing and use in mosquito control programs.

Contributed Paper, Tuesday 9:30 **51**

Investigations into a nematode infecting an exotic invasive ant: new host relationship or hitchhiker across the Atlantic?

Eleanor Groden[1], S. Patricia Stock[2], Robert Lopez[2], and Jennifer Lund[1]

[1] School of Biology and Ecology, University of Maine, Orono ME 04469, USA; [2] Department of Entomology, University of Arizona, Tucson AZ 85721, USA.

Address for correspondence: eleanor.groden@umit.maine.edu

Myrmica rubra (Linn.), is a northern temperate ant common in moist soils in its native range in Europe and western Asia. Although first reported in the U.S. in 1908, and confirmed in Maine in 1952, this ant has been rapidly spreading and become locally dense and pestiferous over the past 10-15 years in Maine, neighboring states, and Canadian provinces. We first observed nematodes emerging from ants collected at two sites in Maine in 2003 and 2004, and collected a similar diplogasterid-like nematode in ants in England in 2003. These nematodes were not maintained alive and further identification was not possible. In July and August 2008, *M. rubra* colonies collected from seven sites in Maine were parasitized with nematodes, and affected colonies collapsed. Nematodes from each site were maintained separately, reared on baby-food agar and *Galleria mellonella* larvae, and used to challenge *M. rubra* colonies. Ant mortality varied with the site of origin of the nematode, ranging from 2.5% (not different from control) to 27.3% 18 days post exposure. Nematode populations were identified with a combination of molecular and morphological methods. Sequences were generated from the PCR amplicons derived with nematode 18S (barcode) and 28SrDNA primers for molecular characterization, and DIC microscopy was used to depict key morphological traits to confirm molecular identification. Nematodes from most sites were confirmed as members of the family Diplogasteridae belonging to the genus *Pristionchus*, specifically the species *P. entomophagus*, which is known as a necromenic associate of scarab beetles. Infection mechanisms will be considered.

Contributed Paper, Tuesday 9:45 **52**

Scavenger Deterrent Factor (SDF) from Symbiotic Bacteria of Entomopathogenic Nematodes

Selcuk Hazir[1], Baris Gulcu[2] and Harry K. Kaya[3]

[1]Adnan Menderes University, Faculty of Arts and Sciences, Department of Biology, Aydin, Turkey; [2] Duzce University, Faculty of Arts and Sciences, Department of Biology, Duzce, Turkey; [3]Department of Nematology, University of California, One Shields Avenue, Davis, CA 95616

Addresses for correspondence: selcukhazir@gmail.com

Entomopathogenic nematodes (EPNs) in the genera *Steinernema* and *Heterorhabditis* are symbiotically associated with bacteria *Xenorhabdus* spp. and *Photorhabdus* spp., respectively. In an earlier study, the symbiotic bacteria produced chemical(s) that deterred ants from feeding on nematode-killed insects; the chemical(s) was called an Ant Deterrent Factor (ADF). We studied the response of different scavengers (ant, cricket, wasp and calliphorid fly) to ADF. These scavengers' response to nematode-killed insects and to *Photorhabdus luminescens* cultures of different ages was evaluated. Ants, crickets and wasps fed on 1-day-old monoxenic nematode-killed and frozen-killed insects. Crickets consumed 2- to 7-day-old axenic nematode-killed insects, 1- to 5-day-old insects killed by the entomopathogenic bacterium, *Serratia marcencens*, and frozen-killed 1- to 10-day-old insects that were allowed to putrefy. All scavengers did not feed on nematode-killed insects containing both the nematode and the symbiotic bacterium that were more than 2 days old. In the *P. luminescens* experiments, ants fed only on 5% sucrose solution and 24 - 72 h *P. luminescens* cultures that were 24- to 384-h old containing 5% sucrose. Wasps showed no interest to meat soaked in *P. luminescens* supernatant, whereas they fed on meat soaked in *Escherichia coli* supernatant and control meat. Lyophilized *P. luminescens* supernatant soaked in meat showed the same "no interest" response by the wasps. Calliphorid flies did not oviposit on meat soaked in *P. luminescens* supernatant but did oviposit on control meat. Based on the response of these scavengers, we recommend that the chemical compound(s) responsible for this deterrent activity be called "scavenger deterrent factor" (SDF).

Contributed Papers Tuesday, 8:00-10:00

Viruses 3

Contributed Paper, Tuesday 8:00 **53 STU**

IE0 coupled with low levels of IE1 enables rapid DNA replication in Autographa californica multiple nucleopolyhedrovirus (AcMNPV): Rationale for the requirement of IE0 and IE1 to achieve wildtype infection

Nadia R. Sokal[1]; Yingchao Nie[2]; Leslie G. Willis[2]; Mark Rheault[1]; David A. Theilmann[1,2]

[1]Dept. of Biology, University of British Columbia, Okanagan, [2]Pacific Agri-Food Research Centre, Agriculture and Agri-Food Canada, Box 5000, Summerland , B.C., Canada V0H 1Z0

Address for correspondence: david.theilmann@agr.gc.ca

The exact functions of the two primary trans-regulatory proteins, IE0 and IE1, in alphabaculovirus AcMNPV DNA replication are unknown. Both IE0 and IE1 are expressed throughout the viral life cycle, but at different levels, and both are involved in regulating viral DNA replication and transcription. Either protein can support virus replication but both are required to achieve a wildtype infection. If only IE0 or IE1 are expressed during virus infection different viral phenotypes are observed suggesting different roles for each of these trans-regulators. Different phenotypes previously observed may result, at least partially, from different levels of gene expression rather than functional differences of the proteins. In this study identical promoters were used to achieve similar levels of expression of either *ie0*, *ie0*$^{M\text{-}A}$ or *ie1*. Expression of *ie0* results in the translation of both IE0 and a small amount of IE1, and expression of *ie0*$^{M\text{-}A}$ results in only IE0 being produced. Viruses were constructed that expressed *ie0*, *ie0*$^{M\text{-}A}$ or *ie1* under control of either the *ie1* or *gp64* promoter. All constructs, including those using the *gp64* promoter, were able to support virus DNA replication, BV and ODV production. The native promoter of either *ie0* or *ie1* is therefore not essential for virus replication. The results also show that expression of IE0 results in significantly earlier initiation of viral DNA replication relative to either IE0$^{M\text{-}A}$ or IE1. This suggests IE0 enables rapid initiation of viral DNA replication, however to achieve this but requires co-production of low levels of IE1.

Contributed Paper, Tuesday 8:15 **54**

Deletion analysis of the AcMNPV core gene *ac68* and *ac67* (*lef-3*) shows that the single stranded DNA binding protein LEF-3 is not essential for virus replication

Yingchao Nie[1], Minggang Fang[1], Martin A. Erlandson[2], David A. Theilmann[1]. [1]Pacific Agri-Food Research Centre, Agriculture and Agri-Food Canada, Box 5000, Summerland, B.C., Canada V0H 1Z0; [2]Saskatoon Research Centre, Agriculture and Agri-Food Canada, Saskatoon SK Canada

Address for correspondence: david.theilmann@agr.gc.ca

Using transient assays AcMNPV single-stranded DNA binding protein LEF-3 has been shown to be essential for viral DNA replication and also interacts with the viral helicase P143 enabling

transport to the nucleus. The *lef-3* transcript initiates within the open reading frame of *ac68* which is a baculovirus core gene. Previous deletion analysis of *ac68* resulted in an increase in LT-50 but had no impact on budded virus (BV) production or occlusion body formation. To further understand the function of *lef-3* and *ac68*, a knockout (KO) virus lacking both *lef-3* and *ac68* was made (*lef3-ac68*KO), which was repaired with just *ac68*, *lef-3* or both *lef-3* and *ac68*, to generate *lef-3*KO, *ac68*KO or the *lef3-ac68* double repair virus, respectively. To produce the *ac68*KO without any potential impact on *lef-3*, the *lef3-ac68*KO virus was repaired by the fragment containing *lef-3* and *ac68*, in which *ac68* was mutated so that only LEF-3 can be expressed. All viruses produced occlusion bodies indicating viral DNA replication and late gene expression in the absence of LEF-3 or AC68. Analysis by qPCR surprisingly showed a limited level of viral DNA replication and BV production from *lef3-ac68* double KO and *lef-3*KO. The *ac68*KO produced viral DNA replication levels slightly lower than the double repair or wild type control virus up to 48 hours post transfection. P143 localized to the nucleus in the absence of LEF-3. This study therefore shows that deletion of the core gene *ac68* results in slightly decreased levels of viral DNA replication and BV production. Secondly, it is shown for the first time that even though the loss of LEF-3 severely impairs virus replication it is not absolutely essential for viral DNA replication or for P143 nuclear import.

Contributed Paper, Tuesday 8:30 **55 STU**

ProV-CATH of *Autographa californica* multiple nucleopolyhedrovirus associates with both the chitin-binding and active site domains of the viral chitinase

Jeffrey J. Hodgson[1], Basil M. Arif[2] and Peter J. Krell[1]
[1]Department of Molecular and Cellular Biology, University of Guelph, Guelph, ON N1G 2W1, Canada, [2]Laboratory for Molecular Virology, Great Lakes Forestry Centre, Sault Ste Marie, ON P6A 2E5, Canada
Address for correspondence: jhodgs01@uoguelph.ca

Regulated and coordinated release of virus-encoded chitinase (CHIA) and cathepsin protease (V-CATH) enzymes at the terminal stages of baculovirus replication liquefies the insect larvae aiding efficient insect-to-insect spread of virions. The CHIA C-terminal KDEL motif ensures endoplasmic reticulum (ER) retention of CHIA until cell lysis occurs. The proenzyme form of the viral cathepsin protease (proV-CATH) associates with the viral CHIA in the ER, and is retained in cells due to its interaction with CHIA. Prior literature suggests CHIA assists folding of proV-CATH since it was reported that deletion of *chiA* causes proV-CATH to form insoluble aggregates. CHIA is comprised of two independent domains, the N-terminal chitin-binding domain (CBD) and the C-terminal active site domain (ASD). To see if proV-CATH interacts with the CHIA CBD or ASD and to map the CHIA-proV-CATH interaction sites, we produced viruses that co-express *v-cath* along with either of the truncated *chiA* CBD or ASD. We used mRFP-based bimolecular fluorescence complementation (BiFC) and reciprocal Ni/HIS pull-downs to probe proV-CATH interactions with the CHIA CBD or ASD *in vivo* and *in vitro*, respectively. We detected BiFC fluorescence in the ER due to proV-CATH association with either the CHIA CBD or ASD. The proV-CATH interactions with the CBD and ASD were corroborated biochemically by Ni/HIS pull-downs in which proV-CATH could be co-purified with the CBD or ASD, and vice versa. Although we do not know the actual nature of those interactions, these data indicate that either domain of CHIA is sufficient for proper folding of proV-CATH.

Contributed Paper, Tuesday 8:45 **56 STU**

Characterization of the conserved *chiA* and *v-cath* bidirectional promoter of *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV)

Michael J. Norris[1]; Basil M. Arif[2]; Peter J. Krell[1]
[1]Department of Molecular and Cellular Biology, University of Guelph, Guelph, Ontario N1G 2W1 Canada, [2]Laboratory for Molecular Virology, Great Lakes Forestry Centre, Sault Ste Marie, Ontario P6A 2E5 Canada
Address for correspondence: mnorris@uoguelph.ca

In the AcMNPV genome, ~14% of the genes are arranged divergently on opposite strands with an intergenic region of <1 kbp. In this configuration, a bidirectional promoter generally drives expression of both genes. However, no baculovirus bidirectional promoters have been characterized in any detail. We chose the AcMNPV *chiA*/*v-cath* intergenic region to serve as a model to characterize transcriptional regulation of bidirectional gene pairs during AcMNPV infection. We sequentially truncated putative upstream regulatory regions of *chiA* and *v-cath* to identify sequences essential for transcriptional initiation. Forty bp of the *chiA* gene 5'-flanking region was sufficient to support *chiA* transcription at native levels. Interestingly, *v-cath* transcription from viruses containing only 40 bp of their upstream 5'-flanking region was found to be higher by 4-fold relative to the level of native mRNA expression. Linker-scanning mutagenesis that inserted 5 bp linkers spanning the *chiA*/*v-cath* intergenic region identified nucleotides critical for the transcriptional activation of both genes. From this, nucleotides -33 to -42, of the *v-cath* gene was found to negatively regulate *v-cath* mRNA expression. qRT-PCR studies of native AcMNPV revealed a 2-4 fold higher *chiA* mRNA expression relative to *v-cath* possibly explaining why translation of CHIA can be detected 6 hours earlier than V-CATH. This study identifies upstream regions of viral *chiA* and *v-cath* required for initiation of transcription and provides the first insight into baculovirus mechanisms for transcriptional regulation of interdependent gene pairs.

Contributed Paper, Tuesday 9:00 **57STU**

Analysis of baculovirus auxiliary protein P10 encoded by *Autographa californica* Multiple Nucleopolyhedrovirus (AcMNPV)

Farheen Raza[1]; Caroline Griffiths[1]; Robert D. Possee[2]; Linda King[1]
[1]Insect Virology Research Group, School of Life Sciences, Oxford Brookes University, Oxford, OX3 0BP, UK
[2]Centre for Ecology and Hydrology, Wallingford, OX10 8BB, UK
Address for correspondence: laking@brookes.ac.uk

Baculoviruses require auxiliary proteins to efficiently infect hosts in nature but frequently these proteins are superfluous for viral replication in permissive cultured cells. In *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) the very late auxiliary protein P10 is thought to facilitate cell lysis and aid in the timely release of polyhedra and viral enzymes. We have examined closely the effect of P10 on cell lysis and found that cells infected with a P10 knockout virus remain intact until 240 hpi whereas cells infected with a wild-type AcMNPV undergo lysis from 72hpi. Structurally, P10 displays two morphologies in AcMNPV infected *T.ni* cells; filamentous structures associated with microtubules (MTs) and perinuclear tubules. Sequence analysis of P10 homologues has revealed four potential phosphorylation sites at residues Thr^{19}, Ser^{70}, Ser^{92} and Ser^{93}. Using site-specific mutagenesis, these residues were replaced with alanine and the effect on P10 structures was analysed. Confocal microscopy revealed that filamentous structures from mutated P10 proteins do not differ in their association with MTs compared to wild-type but subtle differences are seen in the perinuclear tubules. Mutation of Ser^{92} and Ser^{93} sites produced aberrant perinuclear tubules

compared to wild-type suggesting a role for these sites in tubule formation.

Contributed Paper, Tuesday 9:15 **58**

Natural point mutations within conserved region II and III of *Autographa californica* multiple nuclepolyhedrovirus DNA polymerase increase drug resistance, affect DNA replication and alter structural morphology of occlusion bodies

Guozhong Feng[1], David Thumbi[2], Basil Arif[3], Daniel Doucet[3] and Peter J. Krell[1]

[1]Department of Molecular and Cellular Biology, University of Guelph, Guelph, ON N1G 2W1; [2]Sylvar Technologies Fredericton, NB E3B 5A6; [3]Great Lakes Forestry Centre Sault Ste Marie, ON P6Q 2E5

Address for correspondence: pkrell@uoguelph.ca

Autographa californica multiple nuclepolyhedrovirus (AcMNPV) DNA polymerase plays an essential role in viral DNA replication. Aphidicolin is a selective viral DNA polymerase inhibitor and abacavir is one of the most efficacious nucleoside analogues with inhibitory activity on reverse transcriptase enzymes. We recovered drug escape AcMNPV mutants derived from the serial passage of the parental AcMNPV in the presence of increasing concentrations of aphidicolin and abacavir. A single (C543R, aphidicolin) or double (C543R and S611T, abacavir) point mutation was found within the regions II and III for these drug resistant isolates. To confirm the role of point mutations in AcMNPV DNA polymerase, a *dnapol* knockout virus was generated using bacmid technology. Furthermore, a repair virus was constructed by transposing the *dnapol* wild-type gene or containing a point mutation into the *polyhedrin* locus of the *dnapol* knockout bacmid. There was a significant increase in resistance of single C543R or double C543R/S611T mutation to both aphidicolin and abacavir. Virus titre analysis and real-time PCR showed the single C543R or double C543R/S611T mutation decreased virus replication, compared to the wild-type repair virus. Surprisingly, Electron-microscopy results revealed the virus with the single C543R mutation led to generation of occlusion bodies with single and multiple enveloped nucleocapsids, while occlusion bodies with only singly enveloped nucleocapsids were found for the virus with the double C543R/S611T mutation. These results demonstrated that the point mutations in AcMNPV DNA polymerase not only increase drug resistance and influence virus replication, but also alter structural morphology of occlusion bodies.

Contributed Paper, Tuesday 9:30 **59**

Construction and characterization of a recombinant AcMNPV with broader host range

Chunfeng Wu, Zihao Deng, Shimao Zhu, Meijin Yuan, Kai Yang, Yi Pang

State Key Laboratory of Biocontrol, Sun Yat-sen University, Guangzhou 510275, China

Address for correspondence: yangkai@mail.sysu.edu.cn

The high host specificity of baculovirus leads to its narrow insecticidal spectrum. Here we show, a recombinant virus, named vAcRev, was constructed and isolated from the Sf9 cells co-transfected with a *p35*-null AcMNPV mutant and partial DNA fragment of SeMNPV. vAcRev can replicate not only in parental virus permissive cell line, such as Sf9, Hi5 and Se301, but also in parental virus nonpermissive cell line SpLi-221 which is derived from *Spodoptera litura*. vAcRev has oral infectivity to *Trichoplusia ni* and *Spodoptera exigua*, but not to *S. litura*; however, it has hemocoelic infectivity to *S. litura*. Genome sequencing showed that the isolate contains two genotypes.

Contributed Paper, Tuesday 9:45 **60**

Construction of a recombinant *Autographa californica* nucleopolyhedrovirus without using cell culture

Kaoru Teduka, Yasuhisa Kunimi, Ayako Hirao and Madoka Nakai

Tokyo University of Agriculture and Technology, Saiwai-cho, Fuchu-shi, Tokyo 183-8509, Japan.

Address for correspondence: madoka@cc.tuat.ac.jp

Construction of recombinant viruses is important for elucidating viral gene function and infection processes, as well as for recombinant protein production. However, with the exception of a few model systems including *Autographa californica* nucleopolyhedrovirus (AcMNPV), most of the ~600 known baculoviruses lack permissive cell lines in which recombinant genotypes can be produced and, consequently, the molecular pathology of those viruses is poorly understood. The purpose of this study was to establish a generally applicable methodology to construct recombinant baculoviruses without having to use cell culture. We chose AcMNPV as a model virus to compare an *in vivo* system using noctuid larvae and an *in vitro* system using Sf9 cells. The lacZ marker was used to identify recombinant viruses, in conjunction with beta-galactosidase assay and confirmation by PCR. Compared to an initial recombinant genotype frequency of 25% in Sf9 cells, *in vivo* recombination using *Spodoptera exigua* larvae yielded the lower recombinant frequency of 2%. *In vivo* isolation was carried out by injecting low concentrations of hemolymph-derived budded viruses into the hemocoel of uninfected larvae. After four passages at less than LC_5 (5% lethal concentration), the proportion of recombinant AcMNPV in infected larvae reached almost 100%. This study shows that it is feasible to construct recombinant baculovirus without using cell cultures.

Contributed Papers Tuesday, 8:00-10:00

Microbial Control 2

Contributed Paper, Tuesday 8:00 **61**

Progress in the development of two novel microorganisms as bioinsecticides for control of sucking and chewing insect pests

Timothy B. Johnson, Lisa Chanbusarakum and Pamela Marrone

Marrone Bio Innovations, 2121 Second Street, Suite 107B, Davis, CA 95618

Address for correspondence: tjohnson@marronebio.com

Two novel microorganisms, *Chromobacterium subtsugae* and a novel isolate of the genus *Burkholderia*, have previously been identified as possessing insecticidal activity in laboratory bioassays. In addition to demonstrating toxicity to Lepidoptera larvae, fermentation broths of both species also exhibit bioactivity against certain sucking insects and mites. Initial field experiments in 2010 were conducted to determine effective rates, application frequency and range of species controlled. Liquid formulations of fermentation broths from both species demonstrated the capability of providing effective control of several important species including western flower thrips, *Frankliniella occidentalis*, potato psyllid, *Bactericera cockerelli*, beet armyworm, *Spodoptera exigua*, and diamondback moth, *Plutella xylostella*. Challenges in the production and formulation of both species will be discussed.

Contributed Paper, Tuesday 8:15 **62**

Field effectiveness, environmental safety and characteristics of a new isolate of *Metarhizium anisopliae* derived from soil in Alberta, Canada

Dan L. Johnson

Water and Environmental Science Building, University of Lethbridge, 4401 University Drive, Lethbridge, Alberta, T1K 3M4Canada

Address for correspondence: dan.johnson@uleth.ca

Isolates of *Metarhizium anisopliae* were derived from soil in Alberta, Canada, during 2006 and 2009, using selective microbial medium. One isolate, "S54", is proposed for licensing in Canada as a pest control product. In laboratory tests, it proved to be highly infective to grasshoppers (Orthoptera: Acrididae), resulting in nearly 100% mortality within one 7 to 9 days. In other laboratory tests, it was highly infective to other insect pest species. Adult grasshoppers are killed equally as well as immature stages. Operational-scale field tests were conducted in lentils in Saskatchewan and on pasture in Alberta. Spores were applied to replicated plots (each 1.0 to 1.5 ha, replicate blocks and years) at 25 or 50 g per ha, in vegetable oil emulsion, using a standard flat-fan nozzle field sprayer. Application of 50 g/ha resulted in 74.8% reduction in target insects in 6 days, compared to 95.4% for Lorsban (Abbotts adjusted mortality). At day 15, apparent effectiveness of the Lorsban was 78.8%, and 83% in the 50 g/ha *Metarhizium* plots. At 25 g/ha, apparent mortality was less but not significantly different. Reduced activity of survivors was observed. Production and storage tests show that after 8 months of dry storage of conidia, viability remained at 89 to 94%. Collaborative safety tests conducted with rainbow trout, 4 species of aquatic insects, a hymenopteran parasitoid (*Trichomalopsis sarcophagae*), crayfish resident in southern Alberta, a crustacean (*Ceriodaphnia dubia*) and earthworms indicated no adverse non-target effects.

Contributed Paper, Tuesday 8:30 **63**

Efficacy of spot-spray application of *Metarhizium anisopliae* formulated in oil extract of *Calpurnia aurea* in infecting and autodisseminating inoculum amongst adult *Rhipicephalus appendiculatus* ticks in semi-field experiments

N.K. Maniania[1], P. Nana[1], H.I. Boga[2], P. Kamtchouing[3]
[1]International Centre of Insect Physiology and Ecology (*icipe*), PO Box 30772-00100, Nairobi, Kenya; [2]Jomo Kenyatta University of Agriculture and Technology, PO Box 62000-00200, Nairobi, Kenya; [3]Department of Animal Biology and Physiology, University of Yaoundé 1, PO Box 812, Yaoundé, Cameroon
Address for correspondence: nmaniania@icipe.org

Spot-spray application of *Metarhizium anisopliae* formulated in oil extract of *Calpurnia aurea* was evaluated for attraction, infection and dissemination of inoculum among *Rhipicephalus appendiculatus* ticks in semi-field experiments. Formulation was sprayed on 900-cm^2 spot using a hand sprayer. Ticks were released from 4-5 m and were attracted to the treated spot. Half of the ticks collected were maintained in the laboratory while the other half was exposed to rabbits in a ratio of 1:1 male to female to evaluate the effect of fungal infection on feeding and reproduction potential of female ticks. Eighty-three percent of ticks that were brought to the lab died from fungal infection while 40% of the ones on rabbit. The engorgement period of fungus-infected ticks significantly increased by 16% compared to the control. A significant reduction in body weight, egg-mass and hatchability of eggs produced by surviving fungus-infected female ticks was recorded. Eggs that failed to hatch and incubated developed mycosis on the surface. The study also investigated the horizontal transmission of the inoculum among the ticks. Male ticks were infected as above and introduced in plastic bucket containing grass. Uncontaminated female was later introduced in the bucket in a proportion of 1:1. Buckets were maintained for 5 weeks in field conditions. More male ticks (95%) were recovered from the control than from *M. anisopliae* (53%) treatments 5 weeks post-inoculation. Fifty-six percent of females died from fungal infection, indicating a transfer of inoculum from infected males to healthy females during either copulation or casual contacts.

Contributed Paper, Tuesday 8:45 **64**

Field suppression of the mango seed weevil, *Sternochetus mangiferae* with two formulations of *Metarhizium anisopliae* on mango orchard

Sunday Ekesi and Nguya K. Maniania
International Centre of Insect Physiology and Ecology (*icipe*), P Box 30772-00100, GPO, Nairobi, Kenya.
Address for correspondence: sekesi@icipe.org

The mango seed weevil (MSW) *Sternochetus mangiferae* (Coleoptera: Curculionidae) is an important pest of mango in different parts of Africa. Direct damage by the pest can range from 30-72% depending on the cultivar, locality and season. In addition to the direct damage, indirect damage is associated with quarantine restrictions that limit export of mangoes to large lucrative markets in Europe, the Middle East, Japan and USA. The use of synthetic chemical insecticides to control this pest has been unsuccessful. There is therefore the need to look for alternatives management method. In previous studies, we screened 17 isolates of *Metarhizium anisopliae* and *Beauveria bassiana* against adult MSW and identified *M. anisopliae* isolate ICIPE 62 as the most virulent. In the current study, we evaluated field performance of this isolate for the management of MSW on mango. Emulsifiable and aqueous formulations of fungal conidia were applied three times each to the mango trunk at the concentration of 1 x 10^{12} conidia ha^{-1}. Compared with untreated control orchard, both formulations significantly reduce MSW infestation in fruits. However, the emulsifiable formulation was superior to the aqueous formulation in reducing fruit infestation. No significant difference in fruit infestation was found between plots treated with emulsifiable formulation of the fungus and the synthetic insecticide treatment. Adult insects collected from the fungus-treated orchard and reared in the laboratory showed high mortality due to mycosis. The results of these experiments suggest that *M. anisopliae* isolate ICIPE 62 is a potential candidate for the management of MSW on mango.

Contributed Paper, Tuesday 9:00 **65**

Evaluation of fungal entomopathogens for management of chilli thrips, *Scirtothrips dorsalis* Hood, on pepper

Steven Arthurs[1]; Luis Aristizabal[1], Pasco Avery[2]
[1]Mid-Florida Research and Education Center, University of Florida, 2725 Binion Rd, Apopka, FL 32703, USA; [2]University of Florida, Indian River Research and Education Center, 2199 South Rock Road, Fort Pierce, FL 34945
Address for correspondence: spa@ufl.edu

Chilli thrips, *Scirtothrips dorsalis* Hood (Thysanoptera: Thripidae), is an invasive pest in the Caribbean, Florida and Texas. Exclusive reliance on insecticide applications is not a sustainable option for *S. dorsalis*, due to high costs, risks of pesticide resistance, and adverse effects on beneficial organisms and the environment. We evaluated three mycoinsecticides [PFR-97 WDG formulation (AI *Isaria fumosorosea* strain Apopka 97), Botanigard 22WP (AI *Beauveria bassiana* strain GHA) and Met 52 EC (AI *Metarhizium brunneum* strain F52)] for activity against *S. dorsalis* based on concentration-response leaf disc assays and greenhouse tests with potted bell pepper cv. California Wonder. Laboratory tests showed that LC_{50} for adult thrips was between 10^5 - 10^7 conidia or blastospores per ml for adult thrips but higher (10^6 – 10^8 conidia or blastospores per ml) for thrips larvae (ostensibly due to moulting). In greenhouse tests over 5 weeks, all treatments significantly reduced thrips numbers within two weeks of spraying. Spinosad insecticide (Conserve) was the most effective treatment, with >99% control of adults and larvae wrt controls. All three microbial treatments and a highly refined paraffinic oil (SuffOil-X) reduced both adult and larval thrips by >80%. There were also increases in plant and fruit weights and the number of marketable fruits in the Met52, SuffOil-X and spinosad treatments at the end

of the study. These data show that mycoinsecticides have potential in management strategies for chilli thrips.

Contributed Paper, Tuesday 9:15 **66**

Optimizing and integrating microbial biocontrol strategies for western flower thrips on chrysanthemums

Michael Brownbridge, Taro Saito, L. Patrick Schenck, Rose Buitenhuis, Angela Brommit.
Vineland Research and Innovation Centre, 4890 Victoria Avenue N., Box 4000, Vineland Station, Ontario, Canada L0R 2E0
Address for correspondence:
michael.brownbridge@vinelandresearch.com

Thrips are a major impediment in greenhouse floriculture. They are pests of global significance, resistant to most conventional insecticides and in Canada today, there is only one registered product that will successfully control this insect. Biological control thus offers the only realistic and sustainable option available to Canadian growers. The thrips life cycle provides two distinct environments and life stages that can be targeted with different natural enemies, including microbial biocontrol agents. Above ground, thrips adults and larvae may be controlled with *Beauveria bassiana* (BotaniGard), which is applied as a foliar spray. Nematodes, primarily *Steinernema feltiae* (e.g., Nemasys®) are frequently used against soil-dwelling stages (pro-pupae and pupae). Currently, nematodes are applied by foliar spraying, sprenching, or drenching. We investigated these different techniques and their effects on nematode numbers in the soil and efficacy to define the best and most economical use practices. In addition, a combination treatment consisting of foliar-applied *B. bassiana* and soil-applied (sprench) *S. feltiae* was included in the trial. The individual nematode and fungus treatments effectively suppressed thrips, but the combined nematode/fungus treatment provided superior control, and visible feeding damage on plants receiving the combined treatment was significantly lower. Opportunities therefore exist to enhance the reliability and cost-effectiveness of thrips biocontrol agents by taking an integrated approach to their deployment.

Contributed Paper, Tuesday 9:30 **67**

The use of mycoinsecticides to control pupal stages of western flower thrips

Taro Saito and Michael Brownbridge
Vineland Research and Innovation Centre, 4890 Victoria Avenue N., Box 4000, Vineland Station, Ontario, Canada L0R 2E0
Address for correspondence:
michael.brownbridge@vinelandresearch.com

Consumer demands for pest- and damage-free plants poses challenges to floriculture growers. Western flower thrips (WFT), *Frankliniella occidentalis*, in particular are very difficult to manage due to their cryptic habit, rapid life cycle, and resistance to conventional insecticides. Effective bio-based IPM programs are badly needed. WFT occupies two different habitats during its life cycle (adult and larval stages on foliage, pupal stages in soil). Our research is considering natural enemies which are effective against soil-dwelling stages. Met-52™ Granular Bioinsecticide (containing 9.0×10^8 cfu / g *Metarhizium anisopliae* Strain F52) is registered in Canada for black vine weevil, and BotaniGard® 22 WP (containing 4.4×10^{10} cfu / g *Beauveria bassiana* Strain GHA) is registered for spray application against *F. occidentalis*. The objectives of this study are: 1) to determine the relative efficacy of these mycoinsecticides applied as granular/dry preparations or in suspension (three application rates for each equivalent to: Met-52 0.5, 2.5, 5.0 g/L of dry media; BotaniGard 0.1, 0.2, 0.4 g/L of dry media); and 2) to assess their compatibility with other natural enemies. Pupal survival in Met-52 treated media (all treatments) was significantly lower than the control (ANOVA, $F_{[6, 119]} = 19.87$, $p < 0.001$). The scenario was similar when BotaniGard was used (ANOVA: $F_{[6, 119]} = 7.99$, $p < 0.0001$). A preliminary study of their compatibility with a predatory rove beetle, *Atheta coriaria*, indicates that these mycoinsecticides are marginally safe to adult beetles with approximately 60% survival at the highest rate tested.

Contributed Paper, Tuesday 9:45 **68**

Developing functional biopesticides for the lawn care industry

Michael Brownbridge[1], Taro Saito[1], Pam Charbonneau[2] and Paul Côté[1]
[1]Vineland Research and Innovation Centre, 4890 Victoria Avenue N., Box 4000, Vineland Station, Ontario, Canada, L0R 2E0;
[2]OMAFRA, The Guelph Turfgrass Institute, 328 Victoria Rd. South, Guelph, Ontario, Canada N1L 0H2
Corresponding author:
michael.brownbridge@vinelandresearch.com

The European chafer, *Rhizotrogus majalis*, is the most damaging pest of lawns and amenity turf in Ontario. Larval feeding on fibrous roots of turfgrasses causes serious damage, particularly during spring and early fall. The hairy chinch bug, *Blissus leucopterus*, although more localized, can be equally destructive. Feeding damage by these piercing-sucking insects during hot, dry weather will kill grass when turf is already water-stressed. The lawn-care industry has traditionally used insecticides for their control, but an urban pesticide ban means that effective options are now severely limited. Development of alternative reduced-risk and biological pesticides is an industry priority. The approach taken in the current project aims to provide information in the near-term on best use practices for existing biocontrol agents while providing new candidate organisms or bio-derived materials for insect control that are compliant with the pesticide ban. Chinch bug trials were established in two urban locations in southern Ontario during July/August 2010. Three microbial treatments had a marked effect on chinch populations: a *Metarhizium anisopliae* (Met52®) spray and two nematode (*Steinernema carpocapsae*; Millenium®) treatments; nematode efficacy was significantly enhanced when combined with a botanical wetting agent. Nine treatments were assessed against European chafer larvae in infested turf. Seven weeks after application, grub survival was lowest under a neem/corn gluten granular treatment, nematode (*Heterorhabditis bacteriophora*; Nemasys®G 5 billion/ha) and *M. anisopliae* (Met52) spray treatments. Effects were on par with those obtained using an insecticide standard (Merit®; imidacloprid).

Symposium (Cross Divisional Diseases of Beneficial Invertebrates and Viruses) Tuesday, 10:30-12:30

Viruses of Aquatic Invertebrates

Organizers: Karyn Johnson and Grant Stentiford

Symposium Paper, Tuesday 10:30 **69**

Infectious Myonecrosis: A review of a new virus fisease that emerged in the Americas, was introduced into SE Asia and quickly became OIE listed

Donald Lightner, Bonnie T. Poulos, Carlos R. Pantoja and Rita M. Redman
Dept. of Veterinary Science & Microbiology, University of Arizona, Tucson, AZ 85721, USA
Addresses for correspondence: dvl@u.arizona.edu

Infectious myonecrosis emerged as a significant disease in farm raised *Penaeus vannamei* in 2002 in Piaui State of NE Brazil. By 2006 the disease had spread to six other states in NE Brazil. The infectious nature of the disease was experimentally demonstrated in 2003 and the causative agent was identified as a new virus that was named IMN virus (IMNV). In May 2006, an Indonesian government laboratory located in Situbondo, East Java, reported the occurrence of IMN in farms raising *P. vannamei.* Sequence

analysis of the Indonesian IMNV isolate showed it to be 99.6% identical to the isolate from Brazil, providing molecular support to anecdotal evidence that the virus had been introduced to East Java with *P. vannamei* juveniles or broodstock imported from Brazil. Because of the significant negative impact of IMN on shrimp farm production in NE Brazil, IMN was listed in 2005 as "under study" by the OIE ('Office International des Epizooties' or World Organization for Animal Health). Due to its being transferred to Indonesia and ongoing significant production losses in Brazil, IMN was fully listed by the OIE in 2007. Since 2007, IMN has spread within Indonesia and there are anecdotal reports of the disease in other SE Asian countries. The agent of IMN disease is a an unusual virus in the Family *Totiviridae*. Its closest relatives infect yeast and protozoan parasites including *Saccharomyces cerevisiae*, *Giardia lamblia* and *Leishmania* spp. IMNV has a double-stranded RNA genome of 7560 bp. The virion is icosahedral in shape, 40 nm in diameter and it has a buoyant density of 1.366 g/ml. IMN causes significant disease and mortalities in late postlarvae, juvenile and subadult pond-reared stocks of *P. vannamei*. Other penaeid species have been shown to be infected by the virus, but infection may not result in significant disease. Grossly, affected shrimp present focal to extensive white necrotic areas in the striated muscle, especially of the distal abdominal segments and tail fan that may become necrotic and reddened in some individual shrimp. By histopathology, shrimp with the disease present mixed myonecrosis lesions, often with acute and resolving lesions present in the same shrimp. Acute lesions show coagulative myonecrosis, often with edema, while older lesions are accompanied with hemocytic infiltration and fibrosis. Significant lymphoid organ spheroid formation is typically present, and ectopic lymphoid organ spheroids are often found in the hemocoel and loose connective tissues, especially in the heart lumen and adjacent to antennal gland tubules. In some histological preparations, perinuclear pale basophilic to darkly basophilic inclusion bodies (that are IMNV probe positive) are evident in muscle cells, connective tissue cells, hemocytes, and in cells that comprise lymphoid organ spheroids.

Symposium Paper, Tuesday 11:00 **70**

Evolutionary epidemiology of viral pathogens in shrimp aquaculture

Jeffrey M. Lotz
Department of Coastal Sciences, Gulf Coast Research Laboratory, The University of Southern Mississippi, 703 East Beach Drive Ocean Springs, MS 39564 USA
Address for correspondence: jeff.lotz@usm.edu

Epidemiology is the study of the pattern of disease occurrence. From these patterns, one attempts to infer the risk factors associated with disease outbreaks and to develop strategies that reduce the exposure to risk. Evolutionary epidemiology is epidemiology in the context of evolutionary theory. Of central importance to evolutionary theory is the concept of fitness. Fitness is simply a measure of the number of offspring left to the next generation. For diseases the offspring are newly infected hosts. The genotype with the greatest population growth rate is the fittest and relative population growth rate is synonymous with relative fitness. Natural selection maximizes population growth rate and we can use this tenet to understand the evolutionary trajectory of both hosts and pathogens. Over the past thirty years several highly virulent pathogens have emerged in shrimp aquaculture worldwide. Often, it is assumed that the highly virulent nature of these pathogens results from the recent association of the pathogen with the shrimp and that over time the virulence will diminish and perhaps even vanish. However, recent theories of pathogen evolution conclude that a host-pathogen association does not necessarily evolve steadily toward a more benign state but will evolve to a level that maximizes the fitness of the pathogen. The virulence trajectory depends on the specific life history traits of the host and pathogen. The evolutionary context provides a framework from which predictions of change in the characteristics of the pathogen may be deduced. This context may lead to novel health management strategies.

Symposium Paper, Tuesday 11:30 **71**

Molecular epidemiology of white spot syndrome virus

Just M. Vlak[1]; Dieu T.M Bui[2]; Mark P. Zwart[1,3,4]; Mart C.M. de Jong[4]
[1]Laboratory of Virology, Wageningen University, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands, [2]College of Aquaculture and Fisheries, CanTho University, 3/2 street nr. 1, 008471 Can Tho, Viet Nam, [3]Instituto de Biología Molecular y Celular de Plantas, CSIC-UPV, Ingeniero Fausto Elio s/n, 46022 Valencia, Spain, [4]Quantitative Veterinary Epidemiology, Marijkeweg 40, Wageningen University, 6709 PG Wageningen, The Netherlands
Address for correspondence: just.vlak@wur.nl

White spot syndrome virus (WSSV) is a major disease of shrimp. Since its discovery in the early 1990s in Southeast Asia, the virus spread around the globe in about a decade and has become endemic in penaeid shrimp growing regions. There is no 'quick fix' to control the disease other than rigorous cultivation practices and tight control over the entire production chain. WSSV is mainly transmitted by close contact with diseased individuals but also through virus released in the water. Recently, differences in virulence were found to be associated with aquaculture practices. Therefore, understanding the transmission and epidemiology of the virus and the factors governing virulence may lead to novel intervention or mitigation strategies. WSSV contains a large double-stranded circular DNA molecule and is the sole member of the Genus Whispovirus (family Nimaviridae). A number of virus isolates have been completely sequenced and other isolates have been analysed for the presence of larger or smaller differences. This has led to the identification of regions that are highly variable in sequence including various numbers of repeats and large insertions / deletions spanning up to 13 kilobase pairs. This variation can be used to characterize WSSV isolates and correlate spatiotemporal information of virus isolates and the epidemic status of ponds or farms. With this information models can be built to describe the spread and evolution of WSSV, to explain the epidemiology of the virus in shrimp production systems and to predict the outcome of WSSV infections in shrimp ponds or production regions.

Symposium Paper, Tuesday 12:00 **72**

Evidence for autonomous genetic modification in shrimp and its implications for viral disease diagnosis and control

Vanvimon Saksmerprome [1,2*], Sarocha Jitrakorn[1], Kanokporn Chayaburakul[3], and Timothy W. Flegel [1,2]
[1]Center of Excellence for Shrimp Molecular Biology and Biotechnology (Centex Shrimp), Faculty of Science, Mahidol University, Bangkok 10400, Thailand, [2]National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency (NSTDA), Thailand Science Park, Pathum Thani 12120, Thailand, [3]Department Anatomy, Faculty of Science, Rangsit University, Pathumthani 12000, Thailand
Address for correspondence: sctwf@mahidol.ac.th

It has been hypothesized that random viral inserts occur autonomously in shrimp and lead to specific, hertitable antiviral immunity. To test the prediction regarding random viral inserts, giant tiger shrimp specimens were examined for random genomic insertions of the densovirus IHHNV using PCR analysis with a set of 7 overlapping primer pairs to cover the whole IHHNV genome (~4 kb). PCR failures revealed a high proportion of specimens with

random sequence gaps, suggesting the presence of IHHNV genome fragments only (i.e., possible random genome inserts). Targeting a putative insert from one arbitrarily selected specimen, genome walking revealed a matching viral insert linked to a host microsattelite-like fragment from which a chimeric shrimp/virus primer pair was designed. Use the chimeric primer pair revealed similar insertions in many shrimp specimens, including some infected with IHHNV but showing no signs of disease. In one specimen, 2 related but slightly different inserts were revealed, probably on paired chromosomes. These specimens all gave false positive PCR test results for infectious IHHNV using currently recommended PCR protocols. This is the first experimental support for the hypothesis-based prediction that a random number and length of sequence fragments from a single virus genome commonly occur in the shrimp genome. These inserts can give false positive results for infectious IHHNV with official detection methods used to regulate international seafood trade. In addition, discard of domesticated shrimp breeding stocks based on such false positive results might have negative consequences, if such inserts are related to shrimp antiviral immunity as hypothesized.

Symposium (Microbial Control Division) Tuesday, 10:30-12:30

Microbial Pest Control Agents in IPM Systems

Organizer: Stefan Jaronski

Symposium Paper, Tuesday 10:30 **73**

Introduction: What is IPM anyway?

S. Jaronski (USDA ARS, Sidney MT)

Symposium Paper, Tuesday 10:45 **74**

Microbial Pest Control Agents in Orchard IPM

Lawrence A. Lacey
IP Consulting International, P. O. Box 8338, Yakima, WA 98908, USA
Addresses for correspondence: lerrylacey@yahoo.com

A plethora of insects and mites attack tree fruit crops worldwide. The traditional method for controlling most of these pests is the application of broad-spectrum chemical pesticides. Inundatively and inoculatively applied microbial control agents (virus, bacteria, fungi, and entomopathogenic nematodes) have been developed as alternative means of control for a wide variety of arthropods including orchard pests. Due to their selectivity and safety, microbial control agents are ready made components of IPM systems that do not pose a threat to applicators or the environment and allow other natural enemies to function. In temperate climates the majority of the research and applications in orchards has been conducted in pome fruits, citrus, and stone fruit. Microbial agents can provide effective control of several key orchard pests within an integrated control strategy. Good examples include the use of granulovirus for control of codling moth in apple and entomopathogenic nematodes for control of Diaprepes weevil in citrus. The combined effects of judicious and well-timed applications of softer pesticides, such as spinosad, microbial control agents and habitat manipulation to encourage predator and parasitoid species have been successfully employed in organically certified orchards. The challenge will be to find successful combinations of these integrated control components for conventional orchards.

Symposium Paper, Tuesday 11:10 **75**

Managing Tarnished Plant Bug in Southern Row Crops.

O. P. Perera, Gordon Snodgrass, and Ryan Jackson.
Southern Insect Management Research Unit, USDA-ARS, 141 Experiment Station Road, Stoneville, MS 38776

In recent years, the tarnished plant bug (TPB), *Lygus lineolaris*, has become the most significant pest of cotton in the Mid-South. For example, average TPB control cost per acre and yield reduction due to TPB infestations in Alabama, Arkansas, Louisiana, Mississippi, and Tennessee in 2010 was $25.48 and 2.56%, respectively, amounting to 41.7% of the total losses due to insect damage. Currently, chemical insecticides are the only efficient means of controlling TPB, and resistance to insecticides is a major concern. Parasitoids that were successful in other parts of the country failed to yield satisfactory results in the mid-south. Efficacy of fungal and viral entomopathogens are currently being evaluated in field and laboratory settings. Here we discuss the challenges encountered in developing and implementing IPM practices in a highly dynamic cropping system.

Symposium Paper, Tuesday 11:35 **76**

Microbial Control Agents in Greenhouse IPM

Michael Brownbridge
Vineland Research and Innovation Centre, 4890 Victoria Avenue N., Box 4000, Vineland Station, Ontario, Canada L0R 2E0
Address for correspondence:
michael.brownbridge@vinelandresearch.com

Several factors prompted a major shift in greenhouse vegetable pest management in the 1970s and biological control systems are now the 'norm' in vegetable production. Microbials such as *Bacillus thuringiensis* and *Beauveria bassiana* support a suite of predators and parasitoids used to combat recurrent pest species, or are used to control miscellaneous pests when they invade the greenhouse. They are considered highly compatible owing to their limited residual activity and specificity.

Biocontrol is increasingly practiced in floriculture. However, there are challenges to its wider adoption. Recommendations and procedures developed for natural enemies in vegetable crops do not translate directly to ornamentals. Furthermore, ornamentals are valued on their aesthetic appearance; tolerance for cosmetic damage is extremely low. As such, satisfactory levels of control can infrequently be achieved using a single biocontrol agent (the pesticide paradigm). To achieve maximum efficacy in the most cost-effective manner, crop-appropriate use practices and integrated strategies must be devised. Considerable opportunities exist to use microbials, particularly entomopathogenic nematodes and fungi, along with disease-suppressive microorganisms, within bio-based IPM programs. Indeed, they can form the foundation of a successful crop protection strategy where their use is highly complementary to other pest management techniques.

In the long term, greater integration of biocontrol strategies into greenhouse production systems should place growers at a competitive advantage, enabling product differentiation and compliance with key market drivers and consumer demands for pesticide-free plants grown using sustainable practices. In addition, a better working environment is created, pesticide risks reduced and unrestricted access to crops maintained.

Symposium Paper, Tuesday 12:00 **77**

Entomopathogens in small fruit IPM

Surendra K. Dara
University of California Cooperative Extension, San Luis Obispo, CA 93401
Addresses for correspondence: skdara@ucdavis.edu

There are several studies demonstrating the potential of entomopathogens as control agents for a numbr of lepidopteran, coleopteran, hemipteran, homopteran and dipteran pests on small fruits such as blueberries, cranberries, grapes, raspberries, and strawberries. While there are successful examples for using bacteria and viruses, fungal pathogens showed limited efficacy in the field studies and entomopathogenic nematodes are yet to be

exploited. Several strains of entomopathogens are highly pathogenic to many arthropods in the laboratory studies, but their field efficacy widely varies depending on the host, environment, and agronomic practices. Identifying the avenues that can increase the efficacy of the microbial control agents is a key factor for their success and to be a part of IPM. Some examples of microbial pest control agents in small fruit IPM will be discussed.

Contributed Papers Tuesday, 10:30-11:30

Microsporidia 1

Contributed Paper, Tuesday 10:30 **78**

A multi-gene analysis of the microsporidia as eukaryotes

Charles R. Vossbrinck[1]; Heng Xiang[2]
[1]Connecticut Agricultural Experiment Station, 123 Huntington Street, New Haven, CT 06511, [2] Institute of Sericulture and Systems Biology, Southwest University, [2]Tiansheng Rd., Beibei District, Chongqing 400715, China

Early analysis of Microsporidia, based on small subunit ribosomal RNA indicated that they were a very divergent group of eukaryotes. Subsequent analysis using tubulin genes gave a different picture of Microsporidia as the sister taxa to the fungi. Further analysis has apparently pinpointed the Microsporida as Zygomycetes. Numerous genes however, still show the Microsporidia to have sequences which are very different from other eukaryotes. Clearly the Microsporidia are changing at both the nucleotide and protein levels much more rapidly than several of the other eukaryotic kingdoms and affectations such as long branch attraction have to be monitored. At the same time a large number of genes place Microsporida as distant (but perhaps not distantly related) eukaryotes. The relationship between phylogeny and taxonomy needs to be discussed for this group. We analyze a number of genes for the major eukaryotic groups and show the position of the Microsporidia based on Maximum likelihood and Maximum parsimony analysis.

Contributed Paper, Tuesday 10:45 **79**

PCR identification and phylogenetic analysis of the silkworm pathogenic microsporidians, and *Nosema bombycis*

Liu Ji-Ping[1] Li Jin[1] Hao Juan[1,2] Yan De-Hong[1] Zhang Ping[1] Liao Sen-Tai[2]
[1]College of Animal Science, South China Agricultural University, Guangzhou 510642, China
[2]Guangdong Provincial Agricultural Academy Institute, Guangzhou 510640, China
Corresponding author: liujiping@scau.edu.cn

Pebrine disease is a devastating infection that causes massive mortality and losses in silkworm (*Bombyx mori*) colonies that can lead to significant economic losses. Pebrine pathogen is *Nosema bombycis*. As there were discovered several different pathogenic insect microspordians from silkworm and mulberry pests, it is very helpful to develop detection techniques identifying the pathogen *N. bombycis*, for preventing and controlling the incidence and prevalence of pebrine. In this paper, on the basis of collecting, isolating, purifying various kinds of insect microsporidia from the samples of female moths in silkworm fields, according to the putative pseudogene SSUrRNA of *N. bombycis*, PCR primers for detection of most insect microsprodians and species-specific for *N. bombycis* were designed and screened, and the specificity, sensitivity of primers and practicability for production samples were investigated. Furthermore, we simultaneously did clone and sequence analysis of the elongation factor 1-α genes of *N. bombycis* from GuangXi province isolate, *N. Antheraeae* from Shandong province, microsprodian spores from mulberry looper (*Hemerophila atrilineata*) isolate (SCH), and from cabbage butterfly (*Pieris* spp.) isolate (CFD), and from diamondback moth (*Plutella xylostella*) isolate (XCE), respectively. The pseudogenes of SSrDNA of *N. bombycis*（GX）, SCH, CFD were separately amplied and sequenced. All the above fragments were compared and analyzed, The molecular phylogenetic trees of the above two genes were established.The results were showed that SCH, CFD and *N. bombycis* had close relationship, they were located in the clade of *N. bombycis*. *N. antheraeae* is different form *N. bombycis* GX, SCH, CFD and XCE. It was implied that the pebrine pathogens are very diversity, and the existing pathogens were high degree of similarity. So we control the pebrine should control the original and alternative hosts in advance. (Note, this work Supported by the earmarked fund for China Agriculture Research System and by NSFC Grant of 30671588.)

Contributed Paper, Tuesday 11:00 **80 STU**

Nosema spp. goes cellular: the first cell culture model for a honey bee pathogen.

Sebastian Gisder[1], Nadine Möckel[1], Andreas Linde[2], Elke Genersch[1]
[1]Länderinstitut für Bienenkunde Hohen Neuendorf e.V., Friedrich-Engels-Str. 32, D-16540 Hohen Neuendorf, [2]FH Eberswalde, Applied Ecology and Zoology, Alfred-Möller-Str. 1, D-16225 Eberswalde, Germany.
Address of correspondence: elke.genersch@rz.hu-berlin.de

Nosema apis and *Nosema ceranae*, are disease agents of the European honey bee, *Apis mellifera.* Both pathogens, belonging to the phylum microsporidia, are obligate intracellular parasites whose primary targets are the midgut epithelial cells of adult bees. Outbreaks of clinical *Nosema* spp.-infection (nosemosis) are characterized by dysentery and may eventually lead to colony collapse. However, although *N. apis* and *N. ceranae* are important and virulent pathogens of the European honey bee, little is known about cellular or molecular pathogen-host interactions because of the complete lack of a cell culture model so far. Here we present the first cell culture model for both, *N. apis* and *N. ceranae*, based on the infection of the heterologous, permanent lepidopteran cell line IPL-LD-65Y. Using this cell culture model, we were able to document the entire intracellular life cycle of Nosema and observed hitherto undescribed spindle-shaped meronts. Furthermore we developed a RT-PCR-based ELISA detection system using primers which hybridize specific to mRNA of the polar tube protein. With the help of this molecular system we were able to analyze the inhibitory effect of Fumagillin towards *Nosema* spp. in infected cells. This cell culture model provides a previously unavailable means to analyze pathogen-host interactions on molecular and cellular level.

Contributed Paper, Tuesday 11:15 **81**

Genetic diversity of *Dictyocoela*, a feminising microsporidian parasite of crustaceans

Toby J. Wilkinson[1]; Jenny Rock[2]; Nia M. Whiteley[2]; Mykola O. Ovcharenko[3, 4]; Joseph E. Ironside[1]
[1]Institute of Biological, Environmental and Rural Sciences (IBERS), Edward Llwyd Building, Aberystwyth University, Aberystwyth, Ceredigion SY23 3DA, UK. [2]School of Biological Sciences, College of Natural Sciences, Bangor University, Bangor, Gwynedd, LL57 2UW, UK. [3]Witold Stefański Institute of Parasitology of the Polish Academy of Sciences, Twarda street 51/55, 00-818 Warsaw, Poland. [4]Pomeranian Academy, Arciszewski street 22A, 76-200 Słupsk, Poland.
Address for correspondence: jei@aber.ac.uk

Microsporidia of the genus *Dictyocoela* are parasites of gammarid amphipod Crustacea. They typically exhibit low virulence and

efficient vertical transmission and at least some strains are capable of feminising their hosts. Sequencing of a region of the 16S rDNA of *Dictyocoela spp.* from various gammarid host species and localities in Europe and Northern Asia indicates that *Dictyocoela* is genetically diverse and that different strains predominate in different host species. However, the presence of intermediate sequences casts doubt upon previous attempts to describe *Dictyocoela* species on the basis of rDNA divergence alone. Phylogenetic analysis provides little support for coevolution between gammarids and *Dictyocoela*. Furthermore, observations of heavily infected individuals, together with genetic evidence of recombination, suggest that some strains of *Dictyocoela* may be horizontally transmitted and are sexually reproducing. These findings suggest that *Dictyocoela* may be phenotypically, as well as genotypically, diverse, with the potential to exhibit a range of different interactions with its host populations.

WEDNESDAY – 10 August

Symposium (Viruses Division) Wednesday, 8:00-10:00

Pathology of Insect-Virus Interactions

Organizers: Eric Hass-Stapleton and Bryony Bonning

Symposium Paper, Wednesday 8:00 **82**

Rapid transit of AcMNPV by manipulation of the actin cytoskeleton

Taro Ohkawa[1], Aniska Chikhalya[3], Loy E. Volkman[2], Eric J. Haas-Stapleton[3], and Matthew D. Welch[1].
[1]Department of Molecular and Cell Biology and [2]Department of Plant and Microbial Biology, University of California, Berkeley, CA 94720, USA. [3]Department of Biological Sciences, California State University, Long Beach, CA 90840, USA.
Address for correspondence: welch@berkeley.edu

Autographa californica M nucleopolyhedrovirus (AcMNPV) uses intracellular actin-based motility to transit to the nucleus for replication and the cell surface for spread. Motility depends on the viral nucleocapsid protein P78/83, which promotes actin polymerization by activating the actin nucleating Arp2/3 complex. In cells infected with multiple virions, as occurs upon infection of midgut epithelial cells by occlusion derived virus (ODV), a population of nucleocapsids moved to the nucleus and initiated viral early gene expression and a distinct population accumulated in cell surface spikes. Localization to the cell surface required persistent and efficient actin-based motility, as a P78/83 mutant virus that is defective in this process failed to accumulate in surface spikes. Viral accumulation in surface spikes also correlated with the appearance of peripheral actin structures called ventral aggregates, which were previously shown to be induced by the viral early gene Actin Rearrangement Inducing Factor 1 (ARIF-1). An ARIF-1 null mutant did not generate ventral aggregates and showed a reduced percentage of nucleocapsids accumulated in cell surface spikes, indicating ARIF-1 is important for surface localization. Both P78/83 and ARIF-1 mutant viruses also were defective in the infection of orally inoculated *Trichoplusia ni* larvae, demonstrating that defects in actin-based motility and timely cell surface localization impair the ability of AcMNPV to establish infections in primary target tissues. We propose that early virus accumulation at the cell surface involves both actin-based motility and cortical actin rearrangements, and allows pre-replication budding of nucleocapsids to enable rapid spread in the insect host.

Symposium Paper, Wednesday 8:30 **83**

Proteomic studies on BV/ODV phenotypes and implications for baculoviral pathology

Zhihong Hu
State Key laboratory of Virology, Wuhan Institute of Virology, Chinese Academy of Sciences
Address for correspondence: huzh@wh.iov.cn

Baculoviruses have two phenotypes, Budded Virus (BV) and Occlusion Derived Virus (ODV). ODV initiates oral infection in the larval midgut, and BV is responsible for spreading infection within the susceptible tissues. The different functions of BV and ODV are related to their specialized structures. It is generally understood that BV and ODV contain similar nucleocapsids but different envelopes. BV obtains its envelope when budding through cell surface, while ODV acquires its envelope from intranuclear membrane vesicles, which is originally derived from the ER and the nuclear membrane. We employed proteomic techniques to identify protein components of BV and ODV. Isobaric tag for relative and absolute quantification (iTRAQ) and Western blot analyses were used to locate the different proteins in the nucleocapsid and envelope fractions. The results showed that the envelope protein composition of BV and ODV are different. PIFs which are used for oral infection were identified in ODV envelope, while membrane fusion protein F responsible for BV binding and fusion was identified in BV envelope. Apart from the above, other proteins including some baculovirual core proteins were identified in BV and/or ODV envelopes. In comparison, ODV contain many more viral proteins in its envelope than BV. The nucleocapsid composition of BV and ODV revealed the basic structure of the nucleocapsid. Apart from viral encoding proteins, proteomic studies also revealed some host proteins packed within BV/ODVs. Many of the host proteins have been found in other enveloped viruses, indicating enveloped viruses may use common cellular pathways for their infection process.

Symposium Paper, Wednesday 9:00 **84**

Roles of microRNAs in regulating host-virus interactions

Sassan Asgari
School of Biological Sciences, The University of Queensland, St Lucia QLD 4072, Australia.
Address for correspondence: s.asgari@uq.edu.au

MicroRNAs (miRNAs) are small non-coding ribonucleic acids of ~22 nucleotides which play important roles in various biological processes, including development, differentiation, apoptosis and immunity. They are produced in all eukaryotes and some viruses. Accumulating evidence indicates that viral and cellular encoded miRNAs play significant roles in host-virus interactions, including anti-viral responses and tissue tropism targeting the pathogen directly or by altering the expression of host genes that are beneficial to the pathogen. In addition, pathogens may manipulate their host miRNAs to facilitate their replication or entering into latency phase. So far, only a small number of insect virus-encoded miRNAs (an ascovirus, a baculovirus and a nudivirus) and host miRNAs involved in host-virus interaction have been reported.
Latest developments in next generation sequencing platforms and significant reductions in their costs have provided opportunities to study the role of small RNAs, including miRNAs, in host-pathogen interactions. miRNAs can be identified either by targeted approaches (such as cloning) or deep sequencing of small RNAs generated in cells. Comparison of infected versus non-infected hosts using microarray or deep sequencing approaches provides valuable information with regard to identification of differentially expressed host miRNAs upon infection. In addition, virus-encoded miRNAs or small RNAs generated in host cells can be determined. The speaker will discuss utilization of these approaches in the study of miRNAs in a number of host-virus interactions.

Symposium Paper, Wednesday 9:30 **85**

A gut feeling: how baculoviruses establish systemic infections

A. Lorena Passarelli
Ackert Hall, Division of Biology, Kansas State University, Manhattan, Kansas 66506, U.S.A.
Address for correspondence: lpassar@ksu.edu

Many insect viruses and insect-vectored viruses are acquired orally by insects and establish primary infection in the insect midgut epithelium. In the midgut, viruses are physically isolated from accessing other tissues and cells in the hemocoel, and in order to disseminate beyond the midgut, viruses must pass across seemingly unperforated basal laminae that line the midgut. I will describe the alternative midgut escape mechanisms that have been proposed in different insect-virus systems and discuss how *Autographa californica* M nucleopolyhedrovirus (AcMNPV), a baculovirus, and Sindbis virus, a mosquito-transmitted alphavirus, stimulate the activation of basal laminae degrading enzymes to disseminate past the midgut. AcMNPV uses a conserved gene with homology to *fibroblast growth factor*s (*fgf*s) to signal the activation of matrix metalloproteases and effector caspases, which

then allow AcMNPV spread into the hemocoel. Sindbis viruses do not encode *fgf* homologs, but also activate caspases, which enhances their ability to escape midgut barriers. The universality of this mechanism provides insights into curtailing the transmission of vector-transmitted diseases and enhancing the pathogenicity of potential biological insecticides.

Symposium (Mircorposidia Division) Wednesday, 8:30-10:00

Microsporidian-Induced Effects on the Host

Organizers: Dörte Goertz

Symposium Paper, Wednesday 8:30 **86**

Gene expression in ventriculi of bees infected by *Nosema apis* and *Nosema ceranae* Microsporidia

Raquel Martín-Hernández[1]; Aránzazu Meana[2]; Neil Boonham[3]; Mariano Higes[1]

[1]Bee Pathology Laboratory. Centro Apícola Regional.Camino de San Martín s/n, 19180 Marchamalo, Spain, [2]Animal Health Dep., Facultad de Veterinaria, UCM, Avda. Puerta de Hierro s/n, 28040 Madrid, Spain, [3]The Food and Environment Research Agency, Sand Hutton, York, YO41 1LZ, UK.

Address for the correspondence: rmhernandez@jccm.es

Nosemosis is the most widespread of adult bee diseases and causes economic losses to beekeepers (OIE, 2008; Giersch et al., 2009; Heintz et al., 2011). To date, two microsporidian species are related with this disease, infecting honey bees worldwide: *Nosema apis* Zander 1909 and *Nosema ceranae* Fries et al. 1996. Both are intracellular obligate parasites of epithelial cells of the honey bee ventricle. So far, the pathogenic mechanisms in this tissue have not been deeply described. In this work, RNA from ventriculi of bees experimentally infected either by *N. apis* or *N. ceranae* was extracted and the expression of 10 genes related to apoptosis, 6 genes related to cellular cycle, 6 genes related to mitochondrial activity and 2 genes related to hormone levels was studied in cDNA. To do this, specific primers and Taqman® probes were designed. Infection success was determined by RT-PCR in bee ampoules. Four housekeeping genes (GAPDH, EF, B-ACTINE and 18S) were evaluated to select the most appropriate ones for analysis. The efficiency of every reaction was determined to study the gene regulation. The data show an interaction of microsporidia infection and the infected ventricular cells, either in apoptosis response and cell cycle.

Symposium Paper, Wednesday 9:00 **87**

Individual and social pathology of the microsporidian *Nosema ceranae* in the honey bee *Apis mellifera*

Cédric Alaux[1] ; Claudia Dussaubat[1] ; Jean-Luc Brunet[1] ; Cynthia McDonnell[1] ; Raquel Martin-Hernandez[2], Mariano Higes[2] ; Luc Belzunces[1] ; Yves Le Conte[1]

[1] INRA, UMR 406 Abeilles et Environnement, Site Agroparc, 84914 Avignon, France, [2] Bee Pathology Laboratory, Centro Apícola Regional, JCCM, 19180 Marchamalo, Spain

Address for correspondence: cedric.alaux@avignon.inra.fr

In honey bees, nosemosis is a major disease affecting adults and caused by the proliferation of *Nosema* spores in midgut epithelial cells. Recently, *Nosema ceranae*, originally parasitizing the Asian honey bee (*Apis cerana*), has also been found to naturally infect the European honey bee (*A. mellifera*) and is associated with colony losses across the world. However, besides reducing the lifespan of bees, the pathology caused by this microsporidian remains largely unknown. We therefore performed an extensive characterization of *N. ceranae* pathology in worker and queen bees by using molecular, biochemical and chemical analysis.

We showed, at the immunity level, that neither the haemocyte number nor the phenoloxidase activity was affected by *N. ceranae* in our experimental conditions. However, the spore proliferation inhibits in the midgut the transcription of genes involved in cell signalling and tissue integrity and induces oxidative stress. This latter reaction might represent a defence response of the gut epithelium against the infection as found in mammals.

We also demonstrated that *N. ceranae* affects the health of queens, who monopolize the reproduction and regulate the cohesion of the society, by altering their vitellogenin titer (involved in fertility and longevity) and antioxidant capacity. Finally, *N. ceranae* can have an additional impact at the social level since its proliferation significantly alters pheromone synthesis of both workers and the queen. Altogether those results show that besides weakening bees at the individual level, *N. ceranae* can also disturb the colony homoeostasis and provide important insight into honey bee/microsporidian interactions.

Symposium Paper, Wednesday 9:30 **88**

Effect of *Paranosema (Nosema) locustae* (Microsporidia) on behavior and morphological Phase Transformation of *Locusta migratoria manilensis* (Orthoptera:Acrididae)

Wang-Peng Shi

Key Laboratory for Biocontrol of Pests, Ministry of Agriculture, China Agricultural University, Beijing, 100193 China

Addresses for correspondence: wpshi@cau.edu.cn

Infected locusts with *Paranosema locustae* had low antennal sensitivity and aggregation responses to fecal extracts and to *Locusta migratoria manilensis* body volatiles. Infected fifth instar nymphs had significantly lower aggregation index than the uninfected nymphs though with fourth instars the effect on aggregation behaviour only occurred in infected females. Infected adult locusts had significantly lower EAG amplitudes in response to extracts from feces of the adult males. There was no significant difference in the EAG responses between the fifth instar male and female nymphs. Infected nymphs and adults had remarkably low EAG amplitudes for the remaining stimuli in. In the field, spraying *P. locustae* on gregarious locusts caused a substantial population reduction by 16 days after treatment, with most of the surviving locusts being phase solitaria. However, the effects of *P. locustae* on locust phase transformation began before direct mortality had caused a substantial reduction in locust density: locust numbers were still high at day 10, but locusts had already transformed to phase transiens. Laboratory assays showed, at higher dose of 1×10^5 spores/mL, locusts had F/C ratios that were significantly ($P<0.05$) more solitaria than untreated locusts, with locusts having ratios that were either phase solitaria or on the solitaria side of phase transiens. There was no obvious effect of density on female locusts 10 days later as all were were solitaria at all locust densities. At day 16, female locusts were transiens at higher densities, but were solitaria at 4/cage. With males there were lesser effects.

Contributed Papers Wednesday, 8:30-10:00

Bacteria 2

Contributed Paper, Wednesday 8:00 **89**

Analysis of cahderin of *Helicoverpa armigera* as receptor to Cry1Ac

Saad Mousa[1], Patricia Barbosa Pelegrini[2], Swaminathan Sivakumar*[2],Raman Rajagopal[2], Naresh Arora[2], Bindiya Sachdev[2] and Raj K. Bhatnagar[2]

[1]Plant Protection Research Institute, ARC, Dokki, Giza, Egypt

[2]International Centre for Genetic Engineering and Biotechnology (ICGEB), New Delhi, 110 067, India.

Email of corresponding author: raj@icgeb.res.in

B. thuringiensis protein Cry1Ac is the most effective protein in controlling the polyphagous pest *H. armigera*. The receptors of Cry1Ac have been identified as N-aminopeptidases (APN) and cadherins. We have earlier demonstrated that aminopeptidases from *H. armigera* (HaAPN1) function as receptors of Cry1Ac. We

have now cloned and expressed cadherin from *H. armigera* through baculovirus. The domain of cadherin, facilitating toxicity has been mapped. The interaction between the expressed cadherin and Cry1Ac was demonstrated by ligand blot, pull down assays and by chromatography procedures. The functional role of cadherin as a mediating-Cry1Ac toxicity-receptor was evaluated by knocking down its expression in larvae by RNAi. The decrease of cadherin transcription in dsRNA-administered larvae correlated to the reduced toxicity towards Cry1Ac. The region of cadherin related to the interaction with Cry1Ac was obtained by Phage Display and oligomerization assays. The results indicated that amino acids at repeat 10 and the membrane-proximal region of cadherin are core-binding regions, functioning as a scaffold for oligomerization of Cry1Ac. Inclusion of cadherin receptor domain facilitating oligomerisation enhances toxicity of Cry1Ac to larvae.

Contributed Paper, Wednesday 8:15 **90 STU**

Characterization of the Cry1Ac-induced midgut regenerative response to intoxication in *H. virescens* larvae

Anaïs S. Castagnola[1]; Omaththage P. Perera[2]; Juan Luis Jurat-Fuentes[1]
[1]Department of Entomology and Plant Pathology, University of Tennessee, Knoxville, TN 37996, USA, [2]Southern Insect Management Research Unit, USDA-ARS, Stoneville, MS 38776, USA.
Address for correspondence: jurat@utk.edu

Cry toxins synthesized by *Bacillus thuringiensis* (Bt) are a powerful agricultural tool used to control destructive lepidopteran crop pests, such as *Heliothis virescens*. Although the H. virescens larvae midgut healing response after intoxication with Cry1Ac toxin has been established, the molecular regulation of this process has not been described. This healing response can be studied *in vitro* using primary midgut cell cultures from *H. virescens* larvae as a biologically relevant model. Using this model, we developed a quantitative method to identify midgut cell types in primary cultures and used it to characterize the Cry1Ac-specific midgut regeneration response to intoxication. Using a combined proteomic and genomic approach, we identified secreted proteins involved in the midgut regenerative response and confirmed their participation in the regenerative process using bioassays with *H. virescens* larvae. Our data supports a relevant role for specific hexamerin proteins in promoting midgut regeneration in response to Cry1Ac intoxication. Future applications of this work may include identification of novel targets for development of insecticidal technologies.

Contributed Paper, Wednesday 8:30 **91 STU**

Expression and characterization of five midgut aminopeptidases N from *Ostrinia nubilalis* in Sf21 cells

Cristina M. Crava, Yolanda Bel, Agata Jakubowska, Baltasar Escriche
Department of Genetics, University of Valencia, Dr. Moliner 50, 46100 Burjassot (Valencia), Spain;
Address for correspondence: Baltasar.escriche@uv.es

The lepidopteran aminopeptidases N (APNs) are GPI-anchor enzymes located in the apical membrane of the midgut epithelium cells. These proteins have been broadly studied in different lepidopteran species because their possible role as receptors in the mode of action of the *Bacillus thuringiensis* toxins. More than 80 full length *apn* genes in 20 insect species have been reported, and have been classified in eight different classes by phylogenetic analysis. In order to determine the role of the different APN isoforms, five APNs from larval midguts of the European corn borer (*Ostrinia nubilalis*) belonging to four different classes have been cloned and expressed in an insect cell line (Sf21) by using the Bac-to-Bac baculovirus system. Sf21 cells, which have only residual membrane APN enzymatic activity, were used because insect cell-expressed proteins resemble more their native, functional. In all cases, the infection induced the expression of the cloned APNs which were located in the membrane fraction. Four out of the five expressed proteins showed a molecular weight apparently higher than expected, suggesting the occurrence of insect protein modifications. The activity of these isoenzymes was tested by using neutral (Leu-p-nitroanilide, Ala-p-nitroanilide) and basic (Lys-p-nitroanilide) substrates, as well as, their sensibility to inhibitors (bestatin, actinonin and EDTA) of the APN activity. Each APN showed a specific enzymatic pattern of activity and inhibition suggesting that the APNs expressed in the *O. nubilalis* midgut are not functionally redundant.

Contributed Paper, Wednesday 8:45 **92**

Quantitative Cry1Fa toxin binding analyses using indirect radiolabeling

Siva R. K. Jakka[1], Joel Sheets[2], Ken Narva[2], and Juan L. Jurat-Fuentes[1]
[1]Department of Entomology and Plant Pathology, University of Tennessee, Knoxville, TN 37996; [2]Dow AgroSciences LLC, 9330 Zionsville Road, Indianapolis, IN 46268
Addresses for correspondence: jurat@utk.edu

Binding of Cry1 toxins from *Bacillus thuringiensis* to specific receptors on the insect midgut epithelium is required for toxicity. Analyses of Cry toxin binding to midgut brush border membrane vesicles (BBMV) using radio-iodinated Cry toxins have facilitated the development of toxin binding site models and the characterization of resistance mechanisms involving alterations in Cry toxin receptors. However, quantitative binding studies with Cry1Fa toxin have been restricted due to adverse effects of radio-iodination on the toxin. To address the need for alternative Cry1Fa labeling and binding procedures to facilitate binding studies, we present methodology to indirectly radiolabel Cry1Fa toxin. This labeling method does not affect Cry1Fa toxicity and allows performance of quantitative binding and competition assays.

Contributed Paper, Wednesday 9:00 **93 STU**

Vip3C, a novel class of vegetative insecticidal protein from *Bacillus thuringiensis*

Leopoldo Palma[1,2], C. Sara Hernández-Rodríguez[3], Mireya Maeztu[2], Patricia Hernández-Martínez[3], Iñigo Ruiz de Escudero[1,2], Baltasar Escriche[3], Delia Muñoz[2], Juan Ferré[3], Primitivo Caballero[1,2]
[1]Instituto de Agrobiotecnología, CSIC-UPNA, Gobierno de Navarra, Campus Arrosadía, 31192 Mutilva Baja, Navarra, Spain.
[2]Laboratorio de Entomología Agrícola y Patología de Insectos, Universidad Pública de Navarra, 31006 Pamplona, Spain.
[3]Departamento de Genética, Facultad de CC. Biológicas, Universidad de Valencia, Valencia, Spain
Address for correspondence: pcm92@unavarra.es

Since the identification of the first vegetative insecticidal protein (Vip3Aa1), more than sixty Vip3 toxins have been isolated from *Bacillus thuringiensis* (Bt), increasing the number of potentially useful toxins available for integrated pest control programs and resistance management. In this study, three holotypes of a novel *vip3* gene were identified from different Bt strains and characterized at the molecular level. These genes were 2412 bp long and encode 90-kDa (803 amino acids) proteins with sequence similarities of less than 70% with the Vip3 toxins identified so far. These constitute a novel class of Vip3 proteins, namely Vip3C. The nucleotide sequences of these Vip3C proteins were 99% similar, and differed only in a few point mutations that rendered non-synonymous substitutions in the predicted amino acid sequences. For example, lysine (K) at position 3 in Vip3Ca1 and Vip3Ca3 was replaced by methionine (M) in Vip3Ca2 that modified the predicted secondary structure of the putative signal

peptide from turn (Vip3Ca1 and Vip3Ca3) to alpha-helix (Vip3Ca2). Likewise, threonine (T) at position 215 in Vip3Ca1 and Vip3Ca2 was replaced by alanine (A) in Vip3Ca3. Although this substitution affected the 66-kDa insecticidal fragment, no changes occurred in the predicted secondary structure (alpha-helix). When expressed in *Escherichia coli*, Vip3C proteins showed insecticidal activity against *Chrysodeixis chalcites*, *Helicoverpa armigera*, *Spodoptera exigua*, *S. frugiperda* and *S. littoralis* in preliminary bioassays. Further studies need to be performed to determine the LC50 and the host range of each novel Vip3C toxin.

Contributed Paper, Wednesday 9:15 **94**

The improving of PCR-RFLP identification method of *cry1*-type genes

Dongming Liu[1,3], Changlong Shu[1], Jie Zhang[1], Fuping Song[1], Dafang Huang[2], Jiguo Gao[3]
[1] State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193, China, [2] Biotechnology Research Institute, Chinese Academy of Agricultural Sciences, Beijing 100081, China, [3] College of Life Sciences, Northeast Agricultural University, Harbin 150030, China
Address for correspondence: jzhang@ippcaas.cn

Bacillus thuringiensis is the most widely applied type of microbial pesticides because its high specificity and environmental safety. Primarily insecticidal protein was Insecticidal Crystal proteins (ICPs) which were encoded by *cry* or *cyt* genes. The *cry1*-type genes were useful tools for many species of important lepidopteran pest management; and there were too many *cry1*-type genes been reported. The increasing number of *cry1*-type genes have been defied the currently *cry* gene identify methods. According to the alignment of *cry1*-type genes 5' and 3' ends, we divided *cry1*-type genes into four categories; and four pairs of universal primers were designed respectively. For the restricted number of genes in each category, the *cry1*-type genes can be identified efficiently by PCR-RFLP. To evaluate the new system, we identified *cry1*-type genes in 150 Bt isolates by the current system and the original one. The result showed that the new method has higher resolution than original system, and it can identify many *cry1*-type genes that the original system cannot do. The performance of the new system shows its advantage in *cry1*-type genes identification.

Contributed Paper, Wednesday 9:30 **95**

Genetic diversity of 65 isolates from *Bacillus thuringiensis* using fAFLP

Fernando H. Valicente[1]; Rosane B. da Silva[1]; Thaís B. Rodrigues[2]; André H. C. Mourão[3]; Katia G. B. Boregas[1]; Camila S. F. Souza[3]; Carine G. M. Silva[3]; Emerson C. de Barros[1]; Arthur A. G. Torres[3]; Rodrigo S. Mendonça[3]
[1] Embrapa Maize and Sorghum Research Center, [2] Federal University of Lavras – UFLA, [3]Federal University of São João Del Rei
Address for correspondence: valicent@cnpms.embrapa.br

The genetic variability of *Bacillus thuringiensis* isolates was studied using PCR based technique. Twenty-six isolates identified to the subspecies were used: 9 strains from the USDA (United States Department of Agriculture), 9 from Institut Pasteur and 8 from EMBRAPA, and the other 39 isolates without identification of subspecies also belonging to EMBRAPA were subjected to analysis using fAFLP (Fragment Length Polymorphism using primers with fluorescent dyes), in order to determine the genetic diversity within a group of strains of Bt. Sample of genomic DNA from each isolate was doubled digested with EcoRI and MseI, and the fragments were linked to special adapters. Selective amplification reactions were performed using five primer combinations and PCR products were separated by electrophoresis on an ABI377 sequencer. Genetic distances were obtained using the complement of the Jaccard coefficient, and the groups were performed by the UPGMA method. The five primer combinations generated 495 fragments of which 12 were monomorphic. Out of the twenty-six isolates indentified to subspecies, strain T09 and 344 (*Bt* sp *tolworthi*) showed higher similarity (15%), while isolates HD3-*Bt finitimus* and T24-*Bt neoleonensis* were genetically more distant (92%). Isolates without identification of the subspecies that were collected in the state of Goiás showed greater similarity to form a group with an average distance of 6% and the nearest subspecies to this group was *Bt thuringiensis* (HD2) with 52% similarity. Groups were formed consisting of high similarity with isolates collected in the same state, and others with isolates collected from different regions or states.

Contributed Paper, Wednesday 9:45 **96**

A PCR-RFLP method to condense the *Bacillus thuringiensis* collections

Chunge Zhang[1,3],Changlong Shu[1], Jie Zhang[1], Fuping Song[1], Dafang Huang[2], Jiguo Gao[3]
[1] State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193, China, [2] Biotechnology Research Institute, Chinese Academy of Agricultural Sciences, Beijing 100081, China, [3] College of Life Sciences, Northeast Agricultural University, Harbin 150030, China
Address for correspondence: jzhang@ippcaas.cn

Bacillus thuringiensis is one of the most widely applied microbial pesticides for its high specificity and environmental friendly activity. The toxicity mainly contributed by the Insecticidal Crystal proteins (ICPs), which were encoded by *cry* or *cyt* genes. Currently, the *B. thuringiensis* is the key gene resources for developing genetic modified pest-resistant-crops. Therefore, lots of *B. thuringiensis* screen projects were conducted and yield thousands isolates. Because the natures of current *B. thuringiensis* isolate methods, there were a lot of repeat isolates in the collections. Remove out the repeats may be the most important thing before the pesticidal activity screen and other works. In the present report, we illustrated a PCR-RFLP methods that can arranged the isolates according the *cry* gene they contained, the results shows that the method was a powerful tool to condense the *B. thuringiensis* collections.

Contributed Papers Wednesday, 10:30-12:30

Viruses 4

Contributed Paper, Wednesday 10:30 **97 STU**

The salivary secretome of salivary gland hypertrophy virus-infected tsetse fly *Glossina pallidipes* (Diptera: Glossinidae)

Henry M. Kariithi[1, 3, 5], Agah I. Ince[1, 4], Sjef Boeren[2], Adly M. M. Abd-Alla[3], Monique M. van Oers[1], Aksoy S[6], and Just M. Vlak[1]
[1]Laboratory of Virology, Wageningen University, The Netherlands, [2]Laboratory of Biochemistry, Wageningen University, The Netherlands,[3] Insect Pest Control Laboratory, Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture, Vienna, Austria, [4]Department of Genetics and Bioengineering, Yeditepe University, Istanbul, Turkey. [6]Yale School of Public Health, New Haven, Connecticut, United States of America, [5]Biotechnology Centre, Kenya Agricultural Research Institute, Kaptagat Rd, Loresho, Nairobi, Kenya.
Author for correspondence: just.vlak@wur.nl

Tsetse fly mass rearing is required for the successful application of sterile insect technique (SIT) in fly eradication campaigns. However, these rearings are severely affected by salivary gland hypertrophy virus (SGHV), and mitigation or control strategies are dearly sought. The competence of the tsetse fly *Glossina pallidipes*

(Diptera; Glossinidae) to acquire the virus, support viral replication and maturation, and to successfully transmit the virus is likely to depend on complex interactions between Glossina and viral macromolecules. Critical requisites to SGHV transmission are its replication in salivary gland tissue and the secretion of the virions and/or viral-encoded proteins into the fly's salivary gland cavity. Towards a comprehensive understanding of the factors involved in SGHV infection and transmission, we determined the secretome of the salivary gland of SGHV-infected and non-infected tsetse flies. One-dimensional gel electrophoresis (SDS-PAGE) followed by liquid chromatography with tandem mass spectrometry (LC-MS/MS) and secretome profiles were analyzed with MaxQuant software. Our results show the highest amounts of saliva protein secreted by infected flies dissected 72 h post feed (hpf) relative to noninfected flies. A total of twenty-seven SGHV-encoded proteins were detected in the infected tsetse secretome, eight of which were uniquely expressed at 72 hpf and two at 96 hpf. Additionally, eleven of these twenty-seven proteins have been identified as structural SGHV proteins. Our findings should prove valuable in the identification of potential targets for SGHV mitigation strategies, such as immune intervention, to interrupt the transmission of SGHV in tsetse mass rearing facilities.

Contributed Paper, Wednesday 10:45 **98**

Potential management strategies to suppress Salivary Gland Hypertrophy Virus (SGHV) infection in *G. pallidipes* tsetse flies rearing

Adly M. M. Abd-Alla[a*], Henry Kariithi[a], Andrew G. Parker[a], Bryony C. Bonning[b], Just Vlak[c], Max Bergoin[d], and Marc J.B. Vreysen[a]

[a] Insect Pest Control Laboratory, Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture, Vienna, Austria; [b] Department of Entomology, Iowa State University, Ames, IA 50011, USA; [c]Laboratory of Virology, Wageningen University, Binnenhaven 11, 6709 PD Wageningen, The Netherlands, [d]Laboratoire de Pathologie Comparée, Université Montpellier 2, Place Eugène Bataillon, 34095 Montpellier, France.

Address for correspondence: a.m.m.abd-alla@iaea.org

Many species of tsetse flies (Diptera: Glossinidae) are infected with a virus that causes salivary gland hypertrophy (SGH) and flies with SGH symptoms have a reduced fecundity and fertility. The prevalence of SGH in wild tsetse populations is usually very low (0.2-15.2%). The successful elimination of a *Glossina austeni* population from Unguja Island (Zanzibar) using an area-wide integrated pest management approach with a sterile insect technique (SIT) component (1994-1997), encouraged several African countries to include SIT in their national tsetse control programs. A large facility to produce tsetse flies for SIT application in Ethiopia was inaugurated in 2007. To support this project, a *Glossina pallidipes* colony originating from Ethiopia was successfully established in 1996 at the Insect Pest Control Laboratory of the FAO/IAEA Agriculture & Biotechnology Laboratories in Seibersdorf, Austria, but up to 85% of adult flies displayed symptoms of SGH. As a result, the colony declined and became extinct by 2002. The difficulties experienced with the rearing of *G. pallidipes*, epitomized by the collapse of the *G. pallidipes* colony originating from Ethiopia, indicated the urgent need for a management strategy of the Salivary Gland Hypertrophy Virus (SGHV) for this species The recent sequencing of the virus isolated from *G. pallidipes* (GpSGHV) and studies of the virus transmission modalities allowed to identify suitable management strategies. Three approaches to prevent virus horizontal transmission during blood feeding have been initiated and their preliminary results will be presented. These include 1) modifying the feeding regime currently used; 2) adding to blood specific antibodies against envelope proteins to neutralize SGHV virions released with saliva during feeding process; 3) impeding the SGHV infection using a peptide similar to SGHV005 ORF that is hypothesized to bind to the gut epithelium. Attempts to control

Contributed Paper, Wednesday 11:00 **99 STU**

***Plutella xylostella* larval transcriptome response to *Diadegma semiclausum* parasitism and identification of expressed polydnavirus genes**

Kayvan Etebari[1], Robin Palfreyman[2], David Schlipalius[3], Lars Nielsen[2], Richard V. Glatz[4] and Sassan Asgari[1]

[1]School of Biological Sciences, The University of Queensland, St Lucia QLD 4072 Australia. [2]Australian Institute for Bioengineering and Nanotechnology, The University of Queensland, St Lucia QLD 4072 Australia. [3]AgriScience Queensland, Department of Employment Economic Development and Innovation, Ecosciences Precinct, GPO Box 46, Brisbane 4001, Australia. [4]South Australian Research and Development Institute (SARDI), Entomology, Waite Road, Urrbrae, South Australia, 5064, Australia

Address for correspondence: k.etebari@uq.edu.au

Transcriptomic approaches provide unique opportunities to study insect host-parasitoid interactions at the molecular level. In order to investigate *Plutella xylostella* larval transcriptome response to an ichneumonid wasp *Diadegma semiclausum* parasitism, the host transcriptome profile was analyzed using a short-read deep sequencing method (Illumina). *De novo* assembly of cDNA sequence data generated 172,660 contigs between 100 and10000 bp in length; with 35% of >200 bp in length. Parasitization had significant impacts on 928 identified insect host transcripts. Gene ontology data illustrated that the majority of the differentially expressed genes are involved in binding, catalytic activity, metabolic and cellular processes. In addition, the results show that the transcription levels of antimicrobial peptides, such as gloverin, cecropin E and lysozyme, were up-regulated after parasitism. *D. semiclausum* symbiotic polydnaviruses, known as ichnoviruses, play significant roles in host immune suppression and developmental regulation. In the current study, *D. semiclausum* ichnovirus (*Ds*IV) genes expressed in the host were detected and 19 unique sequences identified from five PDV gene families including vankyrin, viral innexin, repeat elements, a cysteine rich motif, and polar residue rich protein. Vankyrin 1 and repeat element 1 genes showed the highest transcription levels among the *Ds*IV genes.

Contributed Paper, Wednesday 11:15 **100 STU**

Toward understanding of bracovirus mechanism replication

Faustine Louis, Jean Michel Drezen, Annie Bézier, Georges Périquet, Catherine Dupuy

IRBI, Tours, France

Address for correspondence: faustine.louis@etu.univ-tours.fr

A lot of parasitic wasps carry in their genome bracovirus, which replicate in the wasp ovaries to formed viral particles. These particles packaged several dsDNA circles and are injected during oviposition in the haemocoele of the host -typically a lepidopteran larva. In the parasitized host the viral genes expressed are responsible of host physiology alteration enable wasp development. The bracovirus does not replicate in the parasitized host. We can considered that the bracovirus genome is divided in two parts: a "pure" viral part, not encapsidated, constituted of genes implicated in viral production and particles formation which have a Nudiviral origin, and "non-viral' part constituted of numerous segments (which form each circles) encoding mainly for virulence genes of eukaryotic origins and only expressed into the host.

The hypothesis of baculoviral/ nudiviral origin of bracoviruses (Bezier et al, Science 2009), and the sequence of the integrated form of some bracoviruses offers us for the first time the

opportunity to study the mechanism imply in bracovirus replication. How does the bracovirus replicate and has the bracoviruses conserved the replication mechanism of their ancestral nudivirus? With semi quantitative PCR, we had determined the number of replication units, their composition and delimitation. This mapping will allow us to test in southern blot the nature of each replication units and the mechanism employed during viral replication.

Contributed Paper, Wednesday 11:30 **101**

Vankyrin genes of the _Hyposoter didymator_ ichnovirus: transcriptional expression patterns in different host species

Gabriel Clavijo[1,2], Tristan Dorémus[1,2], Marc Ravallec[1,2], Marie-Anne Mannucci[1,2], Véronique Jouan[1,2], Anne-Nathalie Volkoff[1,2] and Isabelle Darboux[1,2]
[1]INRA, UMR 1333, Montpellier, France
[2]Université Montpellier 2, UMR 1333, Montpellier, France
Address for correspondence: darboux@supagro.inra.fr

Polydnaviruses (PDVs) are symbiotic double-stranded DNA viruses produced in the reproductive organs of hymenopteran endoparasitoids and transmitted to their lepidopteran hosts during oviposition. Expression of viral genes in host infected cells and tissues leads to immunosuppression, a condition required for the survival of parasitoids. Recent genomic studies revealed that viral genes are clustered in multigene families in the packaged genome of PDVs. The viral *ankyrin* family is present in all sequenced genomes. Here we identified nine *vankyrin* genes in the genome of the *Hyposoter didymator* ichnovirus (HdIV), the PDV associated with the endoparasitic wasp *H. didymator* (Hymenoptera: Ichneumonidae). *H. didymator* parasitizes several noctuid species, in particular *Spodoptera* spp. Time course gene expression experiments indicate that all HdIV *vankyrins* are detected at moderate and relatively constant level throughout parasitism of *S. frugiperda* larvae. To find out more on tissue and/or species specificity transcriptions, the expression profiles of HdIV *vankyrin* genes were compared between *S. frugiperda and S. littoralis* larvae and in different insect cell lines. Our results showed that, among all *vankyrin* members, *Hd27-vank1* exhibits a highest expression level specifically in all tissues of larvae and cell lines of *Spodoptera*. In contrast, a differential expression pattern and level of HdIV *vankyrins* were observed in two insect cell lines not related to *Spodoptera* species. These results suggest that *Hd27-vank1* is the major *vankyrin* member expressed in *Spodoptera* larvae that has pleiotropic functions during parasitism of *Spodoptera* spp., and that divergence in *vankyrin* gene expression may be related to variation in host specificity.

Contributed Paper, Wednesday 11:45 **102**

A host translation inhibitory factor of _Cotesia plutellae_ bracovirus discriminates mRNAs depending on their dependency on eIF4A

Yonggyun Kim, Surakasi V. Prasad, Rahul P. Hepat
Department of Bioresource Sciences, Andong National University, Korea
Address for correspondence: hosanna@andong.ac.kr

An endoparasitoid wasp, *Cotesia plutellae*, depends on its symbiotic polydnavirus, *C. plutellae* bracovirus (CpBV) for their successful parasitization against the diamondback moth, *Plutella xylostella*. During late parasitization period, there is a marked suppression in host protein levels, at which the wasp larvae grow significantly. Two homologous proteins expressed by CpBV have been identified as host translation inhibitory factors (HTIFs) due to their specific inhibition against host mRNAs. This study discovered an inhibitory target molecule of a HTIF (= CpBV15β) to inhibit specific mRNAs of *P. xylostella*. *In vitro* translation assay using rabbit reticulocyte lysate showed that CpBV15β inhibited translation of a storage protein 1 (SP1) mRNA of *P. xylostella*, but did not HTIF mRNAs. However, the SP1 mRNA deleting 5' UTR was not sensitive to the inhibitory action of CpBV15β. When chimerical mRNAs were prepared by exchanging 5' UTRs of SP1 and HTIF mRNAs, the chimerical SP1 mRNA was translated, but the chimerical HTIF mRNA was not in the presence of CpBV15β. When the secondary structures of both 5' UTRs were analyzed in terms of their thermal stabilities, SP1 mRNA had more stable secondary structure than those of both HTIF mRNAs. When both HTIFs were transiently expressed in nonparasitized *P. xylostella*, they altered the protein patterns located in the plasma. When each five spots were randomly chosen in terms of target or nontarget of HTIF and analyzed by tandem mass and MALDI-TOF, there was a clear difference in their 5' UTR thermal stabilities between these two groups. Based on sequence homology of CpBV15β to eIF4A-interaction domain of eIF4G, immunoprecipitation assays were conducted. CpBV15β antibody pull-downed eIF4A along with CpBV15β in protein extract of parasitized *P. xylostella*. These results suggest that CpBV15β selectively inhibits host mRNAs based on their dependency on eIF4A for their efficient translation.

Contributed Paper, Wednesday 12:00 **103**

The evolution of the largest gene family of bracoviruses

Céline Serbielle [1], Stéphane Dupas [2], Elfie Perdereau [1], François Héricourt[1], Elisabeth Huguet[1], Jean-Michel Drezen[1]
[1]Research Institute on Insect Biology CNRS, University of Tours 37200 Tours, France
[2]Laboratory Evolution Genome and Speciation IRD/CNRS, 91190 Gif-sur-Yvette, France
Address for correspondence: drezen@univ-tours.fr

Gene duplications have been proposed to be the main mechanism involved in genome evolution and in acquisition of new functions. One of the major features of mutualistic viruses associated with parasitoid wasps is the organisation of genes into gene families. The viral genome is integrated in a wasp chromosome as a provirus and virus particles injected in the parasitoids' hosts are essential for parasitism success. The viral genome encodes virulence factors organized in gene families which are required collectively to induce host immune suppression and developmental arrest. We studied the largest gene family which encodes protein tyrosine phosphatases (PTPs) in order to determine how gene family expansion occurred and to identify the evolutionary forces inducing gene copy divergence. Here, we present strong indications that PTP gene family expansion occurred through three main mechanisms; by duplication of large segments of the chromosomally integrated form of the virus sequences (segmental duplication), by tandem duplications within this form and by dispersed duplications. PTP gene copy evolution was shown to undergo conservative evolution along with episodes of adaptive evolution, which were correlated with duplication and wasp speciation processes. Altogether duplications and subsequent gene copy evolution likely contributed to the different patterns of PTP gene expression and activities observed today.

Contributed Paper, Wednesday 12:15 **104**

Integration of bracovirus genome into the host: a prospective gene delivery strategy

Shu Zhang[1]; Kavita Bitra[1]; Markus H. Beck[1]; Michael R. Strand[1]
[1]Department of Entomology, University of Georgia, 120 Cedar Street, Athens, GA 30602, USA.
Address of correspondence: shuzhang@uga.edu

Microplitis demolitor bracovirus (MdBV) is a type of polydnavirus symbiotically associated with the parasitoid wasp *Microplitis demolitor*. Like all polydnaviruses, the encapsidated genome of MdBV is segmented and consists of multiple circular double-stranded DNA. Here we report that most MdBV genomic segments

integrate into the genome of insect host cells. A series of assays collectively suggest that integration is nonrandom. Expression studies further indicate that multiple MdBV genes continue to be expressed following integration.

Symposium (Fungi Division) Wednesday, 10:30-12:30

Fungal Associations with Mites and Ticks

Organizer: Ingeborg Klingen

Symposium Paper, Wednesday 10:30 **105**

From basic pathology to microbial control: on overview of fungal acaropathogens

Leo P.S. van der Geest
Institute for Biodiversity and Ecosystem Dynamics (IBED), University of Amsterdam, P.O. Box 94248, 1090 GE Amsterdam, The Netherlands.
Address for correspondence: lpsvdgeest@casema.nl

The Kingdom Fungi is a monophyletic assemblage with four phyla: Chytridiomycota, Zygomycota, Basidiomycota and Ascomycota and in addition, a group called Deuteromycota, fungi with no known sexual stage. Chytridiomycota represent a primitive group of aquatic fungi with no species that infect mites.The order Entomophthorales (Zygomycota) contains several species (*Neozygites* sp., *Tarichium* sp.) pathogenic to mites. They often have a narrow host range and infect only one or few species of arthropods. They may have a great impact on natural populations of herbivorous mites, but use as biocontrol agents is difficult as mass production in artificial media is not possible. Within the Deuteromycota, the form-class Hyphomycetes is recognised. A well-known genus is *Hirsutella* with about 80 species. Most species are pathogenic to tropical invertebrates, although infections in the temperate climatic zones are also known. Often eriophyids are infected. The fungus may decimate natural rust mite populations, e.g. of *Calacarus heveae* in rubber in Brazil and *Phyllocoptruta oleivora* in citrus in Florida. In a number of instances, *Hirsutella thompsonii* has been a successful biocontrol agent against eriophyid pests. These fungi can easily be mass produced in artificial media. Recently, three *Meira* species have been described from field-collected mites in Israel. No sexual stage is known of these fungi and are therefore grouped in the Deuteromycota. Based on their molecular properties, they should be considered to belong to the Basidiomycota.
Very few Ascomycota have been isolated from mites. These isolations concern mainly Laboulbeniales, small, often minute fungi with a obligate association with arthropods.

Symposium Paper, Wednesday 10:50 **106**

Fungal control of ticks in Europe: Methods and first results

Gernot Walder[1], Hermann Strasser[2], Angelika Prader[3], Roland Zelger[3]
[1] Dept. of Hygiene, Med. Microbiology and Social Medicine, Innsbruck Medical University, Austria, http://www.projekt-ve.it/bekaempfung.php
[2] BIPESCO Team Innsbruck, Institut für Mikrobiologie, Leopold-Franzens Universität; Technikerstraße 25, A-6020 Innsbruck, Österreich, http://BIPESCO.uibk.ac.at/
[3] Land- und Forstwirtschaftliches Versuchszentrum Laimburg, Laimburg 6, I-39051 Pfatten/Auer, Italien, http://www.laimburg.it/

Human and animals are under increasing threat from ticks of the *Ixodes ricinus* complex that have proven been particularly difficult to control. A major problem is their hidden niche and the inherent difficulties of penetrating their habitat with appropriate control agents. In most of the affected areas of agriculture and forest but also in recreation areas in Europe the use of chemical insecticides is undesirable or impossible.

Currently the application of virulent, ecologically competent strains of the insect-pathogenic fungus *Metarhizium anisopliae* appears the best option to control the vector of several nasty bacterial and viral infectious agents. *Metarhizium* is characterised as endemic in pest populations and is said to fulfil the key criteria of BCAs by effectiveness, autodissemination and their excellent persistence. This paper provides first examples of the successful use of *Metarhizium* in the Alpine Regions of Europe. Preventive control approaches based on selective control of tick population size will be discussed.

Symposium Paper, Wednesday 11:10 **107**

Fungal control of ticks in South America

Éverton K. K. Fernandes[1,2], Donald W. Roberts[2] and Vânia R. E. P. Bittencourt[3]
[1] Instituto de Patologia Tropical e Saúde Pública, Universidade Federal de Goiás, Goiânia, GO, Brazil; [2] Department of Biology, Utah State University, Logan, UT, USA; [3] Departamento de Parasitologia Animal, Universidade Federal Rural do Rio de Janeiro, Seropédica, RJ, Brazil.
Address for correspondence: evertonkort@iptsp.ufg.br

Tick biological control offers several advantages over currently available chemical acaricides, including lower costs; but, unfortunately, regulations for microbial pesticides in many South American countries are poorly developed and/or virtually not enforced. Entomopathogenic fungi can cause high mortality in all developmental stages of several tick species, and also reduce subsequent generations due to effects on their reproductive efficacy. In general, field or semi-field trials with water-formulated conidia have shown low control efficacy. The environmental conditions often severely reduce the persistence of fungal conidia in the field; and, thereby, reduce their efficacy. Fungal conidia formulated in oil or in a polymerized cellulose gel have shown more promising results in controlling ticks under field conditions. Although fungal application onto pasture has shown effective results; in consideration of the large area that free-range cattle occupy in most South American countries, it seems that applications directly to animal hosts will be the more economically feasible approach for control of cattle ticks (viz., *Rhipicephalus microplus*). On the other hand, the application of fungi in the field might have an important role in controlling: 1) Ticks species with heteroxenous life cycle, 2) Ticks species that parasitize birds, 3) Tick infestations on animals held in pens, or 4) Ticks infesting wild habitats. In South America, only one commercial product containing fungi as the active ingredient is claimed to control ticks. The exhaustive effort of discovering, developing and improving the quality and efficacy of biological products for tick control should be encouraged, and should be continued until efficient programs are well established and accessible.

Symposium Paper, Wednesday 11:30 **108**

Research priorities for development of effective biological pesticides for control of cattle ticks

Perry Polar
Faculty of Engineering, The University of the West Indies, Saint Augustine, Trinidad
Address for correspondence: perrypolar@gmail.com

There is increasing demand for livestock products particularly in developing countries. Ticks vector microorganisms which cause major diseases in livestock, reduce production of milk and meat and result in approximately US$13.9 -18.7 billion dollars in losses annually. Given that ticks often become resistance to chemical acaricides, biological pesticides are an environmentally friendly option to include in resistance management programmes or organic livestock production. Topically applied fungal based biological pesticides have reduced tick populations in field experiments;

however, there is room for improvement as levels of fungal infection were generally low. A program to develop biological pesticides for the control of cattle ticks should focus three main areas. Firstly, the identifying isolates suitable for commercialization through screening for conidial productivity, persistence, temperature, and tick pathogencity. Secondly, direct impaction, that is, infection caused by contact of conidia in spray droplets with target organisms, may be limited by cattle hair and size of ticks. Developing techniques such as wetting of cattle prior to spraying or cattle dips may improve targeting. Finally, improving persistence of conidia on the cattle surface may improve levels of secondary pick up, that is, fungal infection which occurs as target organisms come into contact with previously applied conidia. Methods which focus measuring persistence of conidia on cattle rather than tick related parameters may allow for more rapid improvements in the technology.

Symposium Paper, Wednesday 11:50 **109**

Overwintering of *Neozygites floridana* and its importance in conservational biological control of spider mites

I. Klingen[1], G. Wærsted[1,2] & K. Westrum[1]
[1] Norwegian Institute for Agricultural and Environmental Research (Bioforsk), Plant Health and Plant Protection Division, Høgskoleveien 7, 1432 Aas, Norway. [2]Norwegian University of Life Sciences, Department of Plant and Environmental Sciences, PO Box 5003, 1432 Aas, Norway.
Address for correspondence: ingeborg.klingen@bioforsk.no

The two spotted spider mite *Tetranychus urticae* is known to overwinter as hibernating females, and these partly inactive females may harbour *Neozygites floridana. N. floridana* is a fungal natural enemy of spider mites. The aim of this study was therefore to investigate whether *N. floridana* may be present inside living hibernating females of *T. urticae* throughout the winter season, and if so, in what prevalence and what stage of its fungal life cycle. Hibernating *T. urticae* females were investigated for the presence of fungal structures throughout one winter (October 12, 2006 to February 19, 2007) in field-grown strawberries in a cold climate in Norway (min. ambient temp -15.3ºC). The study confirmed that *N. floridana* survived the winter as a semilatent hyphal body infection, protected inside live hibernating females. The beneficial fungus *N. floridana* is therefore ready to develop and sporulate as soon as climatic conditions permits, resulting in early season infection of *T. urticae*. An early-season infection of *N. floridana* that may result in the control of *T. urticae* in strawberries is important, since *T. urticae* is known to cause reductions in strawberry yield at much lower population levels in early season than in late season. For *N. floridana* to control *T. urticae* populations early in the spring, factors important for sporulation and dissemination of the fungus needs to be favoured. The adapted use of pesticides, especially fungicides might therefore be very important at this time of the year.

Symposium Paper, Wednesday 12:10 **110**

Factors important for survival and epizootic development of *Neozygites* in spider mite populations

Italo Delalibera Jr[1]; Vitalis Wafula Wekesa[1,2]; Karin Westrum[2]; Vanessa da Silveira Duarte[1]; Thiago Rodrigues de Castro[1]; Silje Stenstad Nilsen[2]; Ingeborg Klingen[2]
[1] ESALQ-Universidade de São Paulo, Brazil; [2] Norwegian Institute for Agricultural and Environmental Research (Bioforsk), Norway.
Address for correspondence: italo@esalq.usp.br

The mite-pathogenic fungus *Neozygites floridana* and *N. tanajoae* are important natural enemies of spider mites and are good candidates for microbial control. To be able to succeed in using *Neozygites* for the microbial control of spider mites we need to understand the factors that affect a *Neozygites* epidemic development. Studies have therefore been conducted with Brazilian and Norwegian isolates of these fungi to reveal the effect of abiotic (temperature, rainfall, humidity, light, pesticides) and biotic factors (host plant, presence of predators) on the fungal performance and epizootic development in cassava green mite, tomato red spider mite and twospotted spider mite populations. For Brazilian isolates, rainfalls do not seem to have an apparent impact on disease progression. Microclimatic humidity seems, however, to be a critical factor. When placed on microscope slides, these two fungi only sporulate at RH≥95%, while *N. floridana*-killed cadavers of the twospotted spider mite placed within the boundary layer of the abaxial side of a leaf sporulated also at 90% RH. The temperature optimum of Brazilian and Norwegian isolates varies and Brazilian isolates can sporulate at temperatures as low as 13°C but the highest production of capilliconidia occurs at 21-25°C. A Norwegian *N. floridana* isolate tested produces the highest numbers of primary conidia at 13°C and 18°C while 23°C resulted in a lower production. Our studies also show that performance of different *N. floridana* isolates may vary with light duration and intensity. In integrated pest management systems, *Neozygites* needs to be compatible with chemical pesticides. Our studies show, however, that several fungicides affect *N. floridana* negatively. Our studies have also shown that performance of *Neozygites* vary with host plants.

Contributed Papers Wednesday, 10:30-11:30

Diseases of Beneficial Invertebrates 1

Contributed Paper, Wednesday 10:30 **111 STU**

Differential expression of immune related genes during bacterial infection in the American Lobster (*Homarus americanus*)

K. Fraser Clark[1, 2]; Adam R. Acorn[1]; Spencer J. Greenwood[1, 3]
[1]Atlantic Veterinary College Lobster Science Centre, [2]Department of Pathology and Microbiology and [3]Department of Biomedical Sciences, University of Prince Edwards Island, Charlottetown P.E.I, C1A 4P3, Canada.
Address for correspondence: fclark@upei.ca

The Canadian lobster fishery is the economic backbone of hundreds of Atlantic Canadian communities and is the most economically significant wild fishery in Canada. Adult American lobsters (*Homarus americanus*, H. Milne Edwards 1837) are resistant to a diverse array of microbial pathogens present in their environment, however they are particularly susceptible to infection by the gram-positive bacterium *Aerococcus viridans* var. *homari*. This pathogen is capable of causing a lethal, systemic infection in *H. americanus* in as few as 4-7 days and represents a significant cause of post-harvest loss. We have examined the genetic mediators of the lobster's immune response during the progression of *A. viridan's* infection using a novel lobster microarray representing over 14,000 genes. Numerous genes are differentially expressed during *A. viridans* infection including many traditionally associated with immune response including: anti-lipopolysaccharide binding proteins, lectins, crustins, alpha-2 macroglobulins, proteases, haemocyanins and a variety of currently unannotated genes. Particular attention has been focused on six anti-lipopolysaccharide binding proteins which share significant levels of sequence homology, but exhibit very different patterns of gene expression. The wealth of gene-specific, and biologically relevant, information generated in this study demonstrates that although genes that share significant sequence homology have similar theoretical function, biological function should always be validated through relevant experimental studies.

Contributed Paper, Wednesday 10:45 **112**

Expression of Cpn60 by *Aerococcus viridans* var. *homari* is associated with virulence during infection of the American Lobster *Homarus americanus*

K. Fraser Clark[1, 2]; Spencer J. Greenwood[1, 3]
[1]Atlantic Veterinary College Lobster Science Centre, [2]Department of Pathology and Microbiology and [3]Department of Biomedical Sciences, University of Prince Edwards Island, Charlottetown P.E.I, C1A 4P3, Canada.
Address for correspondence: fclark@upei.ca

Gaffkemia is a well documented lethal systemic disease of both the American lobster (*Homarus americanus*, H. Milne Edwards 1837) and the European lobster (*Homarus gammarus*, Linnaeus). The causative agent of gaffkemia is the gram positive bacterium *Aerococcus viridans* var. *homari* which is currently present is North American and European waters. Previous studies relying on phenotypic characterization have been unsuccessful at differentiating avirulent from virulent strains of *A. viridans* without conducting lethal *in vivo* trials. Genetic characterization of *A. viridans* strains through 16S rRNA sequencing and RAPD fingerprinting has revealed the presence of at least two subtypes. However, subtype 1 contains virulent and avirulent strains which were genetically identical. The purpose of this study is to determine the proteomic mediators of virulence in *A. viridans*. Quantitative proteomic mapping of these two strains has revealed 29 differentially expressed protein spots, 7 of which are only expressed in the virulent strain and could act as virulence factors. One protein, chaperonin 60, is uniquely expressed in the virulent strain and has been correlated with virulence for many other pathogenic bacteria. The 2D proteomic strategy employed in this study is the first to show phenotypic differences between virulent and avirulent strains. The detection of Cpn60 expression represents a potentially useful tool for identifying the virulent strains of *A. viridans* in epidemiological studies.

Contributed Paper, Wednesday 11:00 **113 STU**

Characterization of bacterial flora in the hemolympyh of juvenile American lobster, *Homarus americanus.*

Daniel Hines[1,2]; Adam R. Acorn[1]; K. Fraser Clark[1,2]; Rémy Rochette[4]; M. John Tremblay[5]; Michel Comeau[6]; Spencer J. Greenwood[1,3]
[1]AVC Lobster Science Centre, [2]Department of Pathology and Microbiology, [3]Department of Biomedical Sciences, Atlantic Veterinary College, University of Prince Edward Island, 550 University Ave. Charlottetown, PEI C1A 4P3 Canada, [4]Biology Department, University of New Brunswick (Saint John), 100 Tucker Park Road, P.O. Box 5050, Saint John, NB, E2L 4L5 Canada, [5] Population Ecology Division, Fisheries and Oceans Canada, Bedford Institute of Oceanography, 1 Challenger Dr., P.O. Box 1006, Dartmouth, NS, B2Y 4A2, Canada, [6]Lobster Section, Fisheries and Oceans Canada, Gulf Fisheries Centre, 343 Université Avenue, PO Box 5030 Moncton, NB, E1C 9B6, Canada.
Address for correspondence: dhines@upei.ca

The American lobster, *Homarus americanus* occurs in high densities in waters throughout Northeastern North America. Through an array of immune defenses, the lobster appears surprisingly resistant to many aquatic pathogens and can efficiently eliminate foreign particles after insult. Despite this, lobsters are at increased risk of exposure to pathogens during perimoult. Juvenile lobster's moult and grow at faster rates than adults, increasing their frequency of exposure to potential infections. In September 2009 hemolymph samples from 25 juvenile lobsters from Young of the Year collectors around PEI, were screened for the presence of bacteria; 60% of individuals were found to be bacteremic. Molecular characterization revealed 7 distinct species, of which 3 were Vibrios. Sampling in September 2010 was carried out in 5 different locations throughout Eastern Canada and Northeastern United States. Of 125 animals, 30% were found to be bacteremic. In many cases, animals were infected with several species of bacteria; mixed infections were subcultured to obtain pure isolates. MALDI-TOF was used to prospectively identify and compare similarities amongst 69 isolates. Using a database of known ionization profiles, 39 isolates were prospectively identified, of which 35 were Vibrios. Mass-Spectroscopy data was used as a similarity measure and separated isolates into 7 major groups. The characterization of bacteria in juvenile lobsters highlights the risk of disease during this demanding life history phase.

Contributed Paper, Wednesday 11:15 **114 STU**

Identification of dysbiotic agents in epizootic shell disease of the American lobster (*Homarus americanus* H. Milne-Edwards, 1837)

Norman J. Meres[1], Cyril C. Ajuzie[2], Masi M. Sikaroodi[1], Jeffrey D. Shields[3], Patrick M. Gillevet[1]
[1]Environmental Science and Policy Department, George Mason University, 4400 University Drive, Fairfax, VA, 22030, [2]Aquaculture, Freshwater and Marine Ecology Research Lab, Applied Fisheries and Hydrobiology Unit, Department of Zoology, University of Jos, Nigeria, [3]Virginia Institute of Marine Science, PO Box 1346, Gloucester Point, VA, 23062.
Address for correspondence: nmeres@gmu.edu

Epizootic Shell Disease in the American Lobster *(Homarus americanus)* is continuing to affect New England populations, with economic consequences to people who depend on the lobster fishery. We hypothesize that a dysbiotic shift in the shell microbial biofilm plays an integral part in the etiology of the disease. Using Multitag Pyrosequencing, we interrogated the community structure of the surface microflora of both healthy and diseased lobsters to correlate the abundance of key taxa with lesions. Discriminant analysis (DA) was applied in this study to identify taxa in the microbial community that are statistically significant in the diseased and healthy states. Of the 170 bacterial operational taxa that were identified, 58 are significant in determining the diseased and healthy states. The remaining 112 are not significantly different in the two states. In addition, the taxon that was identified as the genus *Aquimarina* was present in high abundance in both the healthy and diseased lobsters but with a significantly higher abundance in the diseased state. However, DA analysis demonstrates that this genus may not significantly discriminate the diseased state. Phylogenetic analysis indicates that there is significant diversity of this genus in these samples. We conclude that this is a polymicrobial disease and is more correctly classified as a dysbiosis rather than as one caused by a discrete pathogen.

Cross Divisional Symposium Wednesday, 14:00-16:00

Honouring Lerry Lacey and Harry Kaya

Organizers: Steven Arthurs and Ed Lewis

Symposium Paper, Wednesday 14:00 **115**

Battling codling moth and tuber worms: Lerry Lacey as a postdoc mentor.

Steven Arthurs
Mid-Florida Research and Education Center, University of Florida, 2725 Binion Rd, Apopka, FL 32703, USA
Address for correspondence: spa@ufl.edu

Symposium Paper, Wednesday 14:20 **116**

The real dirt: Harry Kaya's influence on entomopathogenic nematode ecology

Mary E. Barbercheck
Department of Entomology, 501 ASI Building, Penn State University, University Park, PA 16802, USA
Address for correspondence: meb34@psu.edu

During his career, Harry Kaya's research on entomopathogenic nematodes (EPN) has helped to move the field from an early "spray and pray" approach to a more refined approach based on EPN ecology and behavior. His research has sought to elucidate the complexity and heterogeneity of the processes and interactions occurring in the soil and how they affect the use of EPN as biological control agents. Soil is a dynamic system and research by Harry Kaya and his students, post-doctoral scientists, and visiting researchers in his lab have addressed key aspects of the interactions between EPN and abiotic and biotic factors in the environment. Some of these factors have included soil texture and structure and soil water status, and intraguild predation, alternate prey, competition between EPN and other entomopathogens, and other food web interactions. In this presentation, I will review some of the key research conducted or influenced by Harry Kaya that has contributed to our understanding of soil and nematode ecology and their use as biological control agents.

Symposium Paper, Wednesday 14:40 **117**

Lawrence A. Lacey: Colleague, Friend and Born-Again Insect Pathologist

Brian A. Federici
Department of Entomology & Interdepartmental Graduate Program in Cell, Molecular and Developmental Biology, University of California - Riverside, Riverside, California 92521 USA
Address for correspondence: federici@ucr.edu

Symposium Paper, Wednesday 15:00 **118**

Good Scientist, Good Mentor and Good Friend: Harry K. Kaya

Selcuk Hazir
Adnan Menderes University, Faculty of Arts and Sciences, Department of Biology, Aydin, Turkey

Symposium Paper, Wednesday 15:20 **119**

From ecology to application: integrating entomopathogenic nematodes into turfgrass pest management

Albrecht M. Koppenhöfer
Department of Entomology, Rutgers University, New Brunswick, NJ 08901, USA

Symposium Paper, Wednesday 15:20

Autodissemination of Japanese beetle pathogens: from the Azores to Oklahoma

Mike Klein

Contributed Papers Wednesday, 14:00-15:45

Diseases of Beneficial Invertebrates 2

Contributed Paper, Wednesday 14:00 **120**

Transcriptome analysis of the Honey Bee fungal pathogen, *Ascosphaera apis* and implementations to host pathogenesis.

Katherine A. Aronstein[1], Scott Cornman[2], Anna Bennett[3]
[1]Honey Bee Research Unit, USDA-ARS, Weslaco, TX 78596, USA; [2]Honey Bee Research Unit, USDA/ARS, Beltsville, MD Land Grant Program, Kentucky State University, Frankfort, KY 40601, SA. [3]Department of Biology, Georgetown University, Washington, DC 20057
Address for correspondence: Kate.Aronstein@ars.usda.gov

Genome-scale sequence analysis is a powerful and efficient strategy to identify genes involved in the complex interactions between host and pathogen. In this study, we have performed high-throughput sequencing to compare the *Ascosphaer. apis* transcriptome as it is expressed during axenic culture versus controlled infection of the honey bee larvae. This strategy can identify genes that are responsive to the host environment or are otherwise important for pathogenicity. Our data are used to support revised gene models, to evaluate the completeness of the current genome assembly and to identify differential expression between growth conditions. We also identify components of key molecular pathways such as signal transduction and mating type. Our results extend previous genomic analyses of *A. apis*, providing additional resources for investigating pathogenesis and, ultimately, identifying effective strategies for the control and/or prevention of chalkbrood disease in honey bee colonies.

Contributed Paper, Wednesday 14:15 **121**

Honey bee colony collapse in stationary apiaries across the U.S.

Francis Drummond[1]; Kate Aronstein[2]; Brian Eitzner[3]; James Ellis[4]; Jay Evans[5]; Nancy Ostiguy[6]; Walter Sheppard[7]; Marla Spivac[8]; Kirk Visscher[9]
[1]School of Biology, University of Maine, Orono, ME 04469 USA; [2]Honey Bee Research Unit, USDA-ARS-SARC, 2413 E. Hwy 83, Weslaco, TX 78596 USA; [3]Department of Analytical Chemistry, The Connecticut Agricultural Experiment Station, PO Box 1106, New Haven, CT 06504 USA; [4]Department of Entomology & Nematology, Honey Bee Research and Extension Laboratory, P.O. Box 110620, Bldg. 970 Natural Area Drive, University of Florida, Gainesville, FL 32611 USA; [5]USDA Agricultural Research Service, Bee Research Lab, 10300 Baltimore Blvd., Bldg 476, Rm 100, BARC-east, Beltsville, MD 20705 USA; [6]Department of Entomology, Pennsylvania State University, 542 Ag Sciences & Industries Building, University Park, PA 16802 USA; [7]Department of Entomology, Washington State University, Pullman, WA 99164-6382 USA; [8]Department of Entomology, University of Minnesota, 219 Hodson Hall - 1980 Folwell Ave, Saint Paul MN, 55108 USA; [9]Department of Entomology, College of Natural and Agricultural Sciences, University of California, Riverside, CA 92521 USA
Address for correspondence: frank.drummond@umit.maine.edu

Honey bee colony collapse has been hypothesized to be a result of multiple stressors. Previous genomic and proteomic assays of sampled workers have suggested that *Nosema* spp. and both known and undetermined viruses may also be causative agents. In 2009 a research project was initiated that involved apiaries in 7 U.S. states (California, Florida, Minnesota, Maine, Pennsylvania, Texas, and Washington). The apiaries consisted of 30 colonies that were started in April 2009 and 2010 from 3 lb packages and requeened with a narrow genotype population of Italian race queens. Colonies were managed using practices typical for each region, except no practices or medications were used to manage mite parasites, other arthropod pests, and pathogens. Monthly sampling of colony strength, queen status, egg production, pest, parasite, and pathogen prevalence as well as pesticide contamination of wax and pollen was conduced. In addition, climatic data and apiary landscape characteristics were collected. Generalized estimating equations (GEE) were used to assess factors associated with colony death, colony strength through time, and queen supercedure rate. We found significant differences in colony survival rates and supercedure rates among states. A colony level linear model explaining 37.5% of colony death risk suggested that potential factors are: 1) apiary site (representing in part climatic zone, landuse practices related to agricultural production, and pesticide exposure measured from pollen brought back to the colony), 2) prevalence of Israeli Acute Paralysis Virus (IAPV), 3) Varroa mite x apiary site interaction, and 4) *Nosema ceranae* x apiary site interaction.

Contributed Paper, Wednesday 14:30 **122**

Potential for neuro-immune communication in the cricket (*Gryllus texensis*): evidence for an octopamine receptor in hemocytes.

Russell H. Easy & Shelley A. Adamo
Department of Psychology and Neuroscience, Dalhousie University, Canada
Addresses for correspondence: reasy@dal.ca

The biogenic amine octopamine regulates behaviour, mediates the stress response, and modulates immune function in insects. Following an immune challenge octopamine can increase hemocyte phagocytosis and mobility. In fat body octopamine increases the release of energy compounds. Physiological evidence suggests that hemocytes and fat body have receptors for octopamine, and, therefore can respond to octopamine released neurohormonally. To provide molecular evidence that hemocytes and fat body have octopamine receptors, we designed primer sequences based on octopamine receptor sequences in other insects, and identified expression of a putative octopamine receptor using reverse transcription PCR. This is the first molecular evidence of the presence of octopamine receptors in cricket hemocytes. Immunochemistry using antibodies raised against honeybee octopamine receptor (AMOA1) (supplied by B. Smith) confirmed the presence of octopamine receptors in *Gryllus* hemocytes and brain tissue. These data provide further evidence for the presence of octopamine receptors in hemocytes. This observation supports the hypothesis that octopamine can modulate immune function in insects. Ongoing studies include identification of immune genes and measurement of expression during treatment and infection stress. The ultimate goal is to characterize molecules and mechanisms involved in disease susceptibility with a particular interest in the interplay between stress and immunity.

Contributed Paper, Wednesday 14:45 **123 STU**

Sickness behaviour in the Texas field cricket *Gryllus texensis*

Evan R. Fairn; Shelley A. Adamo
Department of Psychology, Dalhousie University, Halifax, NS, Canada B3H 4J1
Address for correspondence: evan.fairn@dal.ca

During an immune response animals can exhibit a suite of behavioural changes ('sickness behaviour') including a decrease in feeding, drinking, locomotion, exploration, and reproduction. These changes are believed to enhance survival of infection, potentially by conserving resources for allocation to the immune response and decreasing exposure to predators when in a debilitated state. Sickness behaviour is well characterized in vertebrates, but studies of invertebrates are scarce. We investigated sickness behaviour in the Texas field cricket *Gryllus texensis*. Immune-challenged (injected with heat-killed *Serratia marcescens* bacteria) crickets fed less than controls but did not differ from controls in locomotive behaviour, water consumption, investment in reproduction, or boldness in a novel environment.

Contributed Paper, Wednesday 15:00 **124 STU**

Chitin-binding proteins of *Paenibacillus larvae* and their role in pathogenesis

E. Garcia-Gonzalez; L. Poppinga; E. Genersch
Länderinstitut für Bienenkunde, Molekulare Mikrobiologie und Bienenkrankenheiten, 16540 Hohen Neuendorf
Address of correspondence: eva.garcia-gonzalez@cms.hu-berlin.de

American foulbrood (AFB) is considered the most contagious and destructive infectious disease in honeybees, caused by the Gram-positive, spore-forming bacterium *Paenibacillus larvae* (Genersch et al., 2006). Despite the growing impact of this disease, molecular mechanisms involved in pathogenesis still remain elusive. It has been shown that *P. larvae* spores ingested by young bee larvae proliferate massively in the midgut lumen and that breaching the epithelium is one of the last steps in the disease process (Yue et al., 2008). However, to achieve their way through the gut, the bacteria must first penetrate the peritrophic matrix, a chitin-rich protective layer of the larval gut. Therefore, we hypothesized that chitin-binding proteins play a major role in both attachment and local degradation of the peritrophic matrix.
Here, we present our data on two chitin-binding proteins secreted by *P. larvae*, which we identified as enhancin and a chitinase-like protein. Knowing that enhancins target insect intestinal mucin (Fang et al., 2009) while chitinases disrupt chitin, which both are the two major components of the peritrophic matrix, we were prompted to functionally characterize them in infected larvae. We show an expression profile during *P. larvae* infection focused on the production of chitinase and enhancin. Transcriptomic, proteomic and histological studies are combined, both *in vivo* and *in vitro*, revealing an important role of these chitin-binding proteins during *P. larvae* infection.

Contributed Paper, Wednesday 15:15 **125**

Infection parameters for *Nosema ceranae* and *Nosema apis* in *Apis mellifera*

Wei-Fone Huang[1]; Yanping Chen[2]; Leellen F. Solter[1]
[1]Illinois Natural History Survey, University of Illinois, 1816 S. Oak St., Champaign, IL 61820; [2] USDA-ARS Bee Research Laboratory, Bldg. 476, BARC-East, Beltsville, MD 20705, USA;
Address for correspondence: wfhuang@illinois.edu

Nosema ceranae, a recently described microsporidian pathogen of the honey bee, *Apis mellifera*, is recognized as a global problem, but there are few clues about the extent of its occurrence or spread prior to 2006. In addition, the nature of its interaction with the familiar congener *Nosema apis* is not well understood and data from laboratories in different countries are conflicting. We compared the development, virulence, infectivity (IC_{50}), spore production, temperature effects and direct competition in the same host of the two pathogens using U.S. isolates and bees. We found no significant difference in virulence between the two species but IC_{50} studies demonstrated that a 5x higher dosage of *N. ceranae* is needed for infection. The host response (mortality) to infection at different temperatures was not significantly different between the two pathogens. At a dosage of 1×10^5 spores per bee (200 x lowest IC_{50} for *N. ceranae)*, *N. ceranae* produced slightly more spores than *N. apis* over the infection period, but when individual bees were inoculated with 1×10^5 spores of each *Nosema* species to produce mixed infections, *N. apis* produced more spores. When viruses were combined with microsporidia at different temperatures, mortality trended higher but was not significantly different from mortality due to microsporidia alone. Different results among laboratories have not been explained, but may be the result of differences in host genotypes or subspecies, or pathogen strains, and the reasons for the apparent replacement of *N. apis* by *N. ceranae* remain largely unknown.

Contributed Paper, Wednesday 15:30 **126 STU**

The expression strategy of the Acheta domesticus densovirus (AdDNV) capsid protein (VP) gene cassette is so far unique among parvoviruses

Kaiyu Liu[1,2], Yi Li[1,2], Françoise-Xavière- Jousset[3], Qian Yu[1], Hanh Thi Pham[1], Jozsef Szelei[1],
François Lépine[1], Max Bergoin[1,3], Peter Tijssen[1]
[1]INRS-Institut Armand-Frappier, Université du Québec, Laval, QC, Canada; [2] currently Central China Normal University, Wuhan 430079 People's Republic of China; [3]Université Montpellier2, Montpellier, 34095 France.
Address for correspondence: peter.tijssen@iaf.inrs.ca

Recently, the cricket industry in North America, supplying food for reptile pets in 6-million American households, has been devastated by AdDNV epizootics (Szelei et al., 2011). The genome of this virus was cloned and sequenced. The transcription map showed that splicing occurred both in the nonstructural (NS) and in the capsid protein (VP) multicistronic RNAs. The VP gene cassette contained two VP ORFs of 597 (ORF-A) and 268 (ORF-B) codons. The VP2 sequence was shown by N-terminal Edman degradation and mass spectrometry to correspond with ORF-A. Mass spectrometry, sequencing and Western blotting of baculovirus-expressed VPs versus native structural proteins demonstrated that the VP1 structural protein was generated by joining ORF-A and B via splicing (II) eliminating the N terminus of VP2. This splicing resulted thus in a nested set of VP1 (816 codons), VP3 (467 codons) and VP4 (429 codons) structural proteins. In contrast, the two introns within ORF-B (Ia and Ib) removed the donor site of splicing II and resulted in the VP2, VP3 and VP4 expression. ORF B may also code for several nonstructural proteins of 268, 233 and 158 codons, respectively. Mass spectrometry demonstrated that other splicing combinations were excluded from generating capsid proteins. The small ORF-B contains the coding sequence for the phospholipase A2 motif, found in VP1 that was shown previously to be critical for cellular uptake of the virus. These splicing features are unique among parvoviruses and define a new genus of ambisense densoviruses. X-ray crystallography of the capsids of this virus is underway.

Contributed Papers Wednesday, 16:30-18:30

Bacteria 1

Contributed Paper, Wednesday 16:30 **127**

Utilization of host microRNAs by *Wolbachia* to regulate host gene expression and facilitate colonization of the dengue vector mosquito *Aedes aegypti*

Mazhar Hussain[1]; Francesca D. Frentiu[1,3]; Luciano A. Moreira[1,2]; Scott L. O'Neill[1,3]; Sassan Asgari[1]

[1]School of Biological Sciences, The University of Queensland, St Lucia QLD 4072, Australia, [2]René Rachou Research Institute – FIOCRUZ, Belo Horizonte MG 30190-002, Brazil, [3]School of Biological Sciences, Monash University, VIC 3800, Australia.

Address for correspondence: s.asgari@uq.edu.au

Wolbachia pipientis is an obligate intracellular symbiont which infects a wide range of invertebrates where they are best known for manipulating host reproduction via cytoplasmic incompatibility or various sex ratio distortions. The *Wolbachia* strain *w*MelPop has been shown to be able to modulate lifespan of host insects and interfere with development of human pathogens in mosquito vectors. However, very little is known about the molecular basis of the interactions. Using microarrays, we show that the microRNA (miRNA) profile of the mosquito, *Aedes aegypti*, is significantly altered by the *w*MelPop-CLA strain of *W. pipientis*. We show that a host miRNA (aae-miR-2940) is induced after *Wolbachia* infection both in mosquitoes and cell lines. One of the targets of aae-miR-2940 was the *Ae. aegypti* metalloprotease gene. Interestingly, expression of the target gene was induced following *Wolbachia* infection, ectopic expression of the miRNA independent of *Wolbachia* or transfection of an artificial mimic of the miRNA into mosquito cells. Silencing of the metalloprotease gene in both *Wolbachia*-infected cells and adult mosquitoes led to a significant reduction in *Wolbachia* density, as did the inhibition of the miRNA in cells. The results indicate that manipulation of the mosquito metalloprotease gene via aae-miR-2940 is crucial for efficient maintenance of the endosymbiont.

Contributed Paper, Wednesday 16:45 **128**

Discovery of a novel protein with activity against Western corn rootworm, *Diabrotica virgifera virgifera*.

Deepa Balasubramanian, Dan Tomso, Brian McNulty, Jill Hinson, Kimberly Sampson, Rong Guo, Jessica Zeigler and Nalini Desai

Bayer CropScience, Research Triangle Park, NC27709

Addresses for correspondence:
deepa.balasubramanian@bayer.com

Western corn rootworm (WCR), *Diabrotica virgifera virgifera*, is one of the most significant pests of corn in the United States and has recently become established in Europe. Although transgenic solutions for WCR control exist, improved management strategies would be highly beneficial in providing greater control of the pest in the field. To obtain novel proteins with high activity and different modes of action against WCR, we surveyed microbial cultures for toxicity against WCR by *in vitro* bioassays. A novel protein with activity against WCR, Arp273, was purified from an active microbial strain by chromatographic techniques. The genome of the active strain was sequenced and gene encoding Arp273 was identified by a combination of mass spectrometry and N-terminal sequencing. Recombinant *E.coli* containing the cloned *arp273* gene was used to purify and test the Arp273 protein which was found be active against WCR. Additionally, Arp273 was not active against other insects indicating that the toxicity is specific to WCR. Transgenic corn events were generated containing *arp273* and these showed good protection against root damage by WCR. Sequence analysis of Arp273 did not reveal homology to known insecticidal toxins suggesting that this protein may act in a novel way to control WCR. Field testing of Arp273 transgenic corn events are in progress to determine if the protein could provide more efficacious control of WCR.

Contributed Paper, Wednesday 17:00 **129**

The occurrence of *Photorhabdus*-like toxin complexes in *Bacillus thuringiensis*

Michael B. Blackburn: Phyllis A. W. Martin; Daniel Kuhar; Robert R. Farrar Jr.;Dawn E. Gundersen-Rindal

Invasive Insect Biocontrol and Behavior Laboratory, Agricultural Research Service, United States Department of Agriculture, Henry A. Wallace Beltsville Agricultural Research Center,Beltsville, Maryland, USA

Address for correspondence: mike.blackburn@ars.usda.gov

Recently, genomic sequencing of a *Bacillus thuringiensis* (*Bt*) isolate from our collection revealed the presence of an operon encoding an insecticidal toxin complex (Tca) similar to that first described from the entomopathogen *Photorhabdus luminescens*. To determine whether these genes are widespread among *Bt* strains, we screened isolates from the collection for the presence of tccC, one of the genes needed for the expression of fully functional toxin complexes. Among 81 isolates chosen to represent relatively abundant biochemical phenotypes, 17 were found to encode a TccC. Phylogenetic analysis of the 81 isolates by multilocus sequence typing revealed that all the isolates possessing a tccC gene were restricted to two sequence types which were most closely related to *Bt* varieties *israelensis*, *morrisoni*, *tenebrionis* and *toumanoffi*. Optical mapping of DNA from *Bt* isolates representing both sequence types revealed nearly identical plasmids which appear to be the location of the tca operon. Relative quantitative real-time PCR-based assays for Tc-encoding *Bt* revealed both tcaA and tcaB genes were expressed within infected gypsy moth larvae.

Contributed Paper, Wednesday 17:15 **130**

3D structure of a novel bacterial toxin complex isolated from *Yersinia entomophaga* MH96 and implications for insecticidal activity

Michael J Landsberg[1], Sandra A Jones[2], Rosalba Rothnagel[1], Sean Marshall[2], Ben Hankamer[1], Mark Hurst[2]

[1] Institute for Molecular Bioscience, The University of Queensland, St Lucia, Queensland, 4072, Australia. [2] Biocontrol & Biosecurity, AgResearch, Lincoln Research Centre, Private Bag 4749 Christchurch, 8140, New Zealand.

Addresses for correspondence: mark.hurst@agresearch.co.nz; m.landsberg@imb.uq.edu.au

Yersinia entomophaga MH96 is a native New Zealand soil bacterium able to cause disease in a wide range of insects. The main disease determinant has been located on a 32-kb pathogenicity island designated PAI_{Ye96}. Residing within PAI_{Ye96} are seven open reading frames that encode an insecticidal toxin complex (Tc), comprising not only the ready-recognized Tc-A, Tc-B and Tc-C components, but also two chitinase proteins (Chi1 and Chi2) that form a composite Tc molecule termed the Yen-Tc. The complex is orally active and has broad insecticidal activity against a range of commercial pests including the diamondback moth (*Plutella xylostella*) and represents the first example of a Tc with associated chitinase activity. Once ingested, the Yen Tc causes massive destruction of the larval gut. A mutation in YenB (Yen-Tc::K9) results in the formation of a sub-complex comprising only the Tc-A and chitinase components. We have assessed the structure of the novel Yen-Tc and its K9 derivative. Comparative single particle electron microscopy (EM) and toxin dose response analyses led to the identification of inactive and active forms of the toxin and allowed the main determinants of toxicity to be mapped. The Tc-A components form the structural basis of a 5-fold symmetric assembly that is substantially different in structure and subunit arrangement to its most well characterized homologue, the *Xenorhabdus nematophila* toxin XptA1. Furthermore, the secreted toxin complex from *Y. entomophaga* MH96 includes two chitinases as an integral part of the complex, a feature not described previously in other ABC toxins.

Contributed Paper, Wednesday 17:30 **131**

Electron-microscopic and genetic characterization of a *Rickettsiella* sp. infecting the manuka beetle, *Pyronota setosa* (Coleoptera: Scarabaeidae)

Regina G. Kleespies[1], Sean D.G. Marshall[2], Christina Schuster[1], Richard J. Townsend[2], Trevor A. Jackson[2], Andreas Leclerque[1]

[1] Julius Kühn Institute (JKI) - Federal Research Centre for Cultivated Plants, Institute for Biological Control, Heinrichstraße 243, 64287 Darmstadt, Germany, [2] AgResearch Limited, Private Bag 4749, Christchurch 8140, New Zealand.

Address for correspondence: Regina.Kleespies@jki.bund.de

Larvae of manuka beetles, *Pyronota* spp. (Coleoptera: Scarabaeidae) cause pasture damage in New Zealand by feeding on the roots of grasses. Surveys for potential biocontrol agents revealed a putative disease, expressed as whitened larvae of one of the outbreak species, *Pyronota setosa*. Microbial diagnosis indicated an intracoelomic, intracellular infection, and a previously unknown intracellular bacterium has been identified with subcellular structures characteristic of infection by *Rickettsiella*-like bacteria. These bacteria were rod-shaped, often slightly bent. Numerous associated protein crystals of variable size and shape occurred within round to oval shaped “giant bodies” either singly or as clusters of smaller crystals. Molecular phylogenetic analysis based on 16S ribosomal demonstrates that the manuka beetle pathogen belongs to the taxonomic genus *Rickettsiella*. Therefore, the pathotype designation `*Rickettsiella pyronotae*´ is proposed to refer to this organism. Moreover, genetic analysis makes it likely that - on the basis of the currently accepted organization of the genus *Rickettsiella* - this new pathotype should be considered a synonym of the nomenclatural type species, *Rickettsiella popilliae*.

Contributed Paper, Wednesday 17:45 **132**

The grape phylloxera and *Pantoea agglomerans* – a perfect match?

Nora C. Lawo; Filomena V. Loiacono; Astrid Forneck

Division of Viticulture and Pomology, Department of Crop Sciences, University of Natural Resources and Life Sciences, Peter Jordan Str. 82, 1190 Vienna, Austria

Address for correspondence: nora.lawo@boku.ac.at

The grape phylloxera (*Daktulosphaira vitipholiae* Fitch, Hemiptera: Phylloxeridae), entered Europe in the middle of the 19th century, finding a susceptible host in European *Vitis vinifera* vine roots. The tiny aphid destroyed millions of hectares within several years. Its spreading could finally be prevented by grafting susceptible European vines onto resistant rootstocks. However, in the last decades more aggressive biotypes were observed on leaf-forming rootstocks and more recently also on European *V. vinifera* vines; thus the need to study this pest in more detail increases. Previous findings showed that the grape phylloxera lack endosymbionts such as *Buchnera* spp., but an association of *Pantoea agglomerans* on leaf-galling forms exists for some aphids in several populations. So far, it remains unclear if *P. agglomerans* has any function for phylloxera. To shed light into this association a greenhouse bioassay was conducted treating either *V. vinifera* cv. Cabernet Sauvignon vines or the rootstock Teleki 5C (*Vitis berlandieri* x *V. riparia*) with a very low (water treatment only), a medium (10^{-7} spores/mL) or a high (10^{-3} spores/mL) *P. agglomerans* concentration. Subsequently, all plants were inoculated with phylloxera eggs. The scope of the bioassay was to assess if phylloxera is more efficient in leaf-gall formation and reproductivity in the presence of a medium *P. agglomerans* concentration on *V. vinifera* compared to vines of the rootstock Teleki 5C. To reflect our findings for natural conditions a field bioassay was conducted. Our studies aimed to gain some insights into the aggressiveness of phylloxera attacking European *V. vinifera* vines.

Contributed Paper, Wednesday 18:00 **133**

***Bacillus thuringiensis* delta-endotoxins activity against nematodes**

Tatiana A. Malinina[1], Ludmila K. Kamenek[1], Valery M. Kamenek[1], Maxim A. Terpilovsky[1]

[1]Ulyanovsk State University, Ecological Department, 42 Lev Tolstoy Street, Ulyanovsk, 432970, Russia

Address for correspondence: tataM17@yandex.ru

Phytoparasitic nematodes cause severe crop damages annually, with losses reaching 90%. In our studies, the free-living nematodes *Turbatrix aceti* turned out to be susceptible to *B. thuringiensis* subsp. *kurstaki* delta-endotoxins. The species of *Anguillulidae* family belong to the Phylum Nematoda, and thus have similar morphology and nutrition. It was shown that the nematodes moved slowly on day 1 of experiment after introducing Bt delta-endotoxins into the medium. The microscopy revealed movement and body shape abnormalities. The symptoms, including tail paralysis, twisting or straightening of the body, caused by an increase in longitudinal muscles tone, were observed. These negative effects were followed by nutrition discontinuation and the death of nematodes. The concentration of toxin 0,6mg/ml caused the death of 90% nematodes. The results suggest that Bt delta-endotoxins possess inhibiting and nematocidal activities. *B. thuringiensis* proved a highly effective and safe to human microbial control agent, active against phytopathogenic microorganisms.

Contributed Paper, Wednesday 18:15 **134**

Monalysin, a novel *β*-pore-forming toxin produced by the by the entomopathogenic bacterium *Pseudomonas entomophila*.

Onya Opota[1]; Isabelle Vallet-Gély[2]; Renaud Vincentelli[3]; Christine Kellenberger[3]; Ioan Iacovache[1]; Manuel Rodrigo Gonzales[1]; Alain Roussel[3]; Françoise-Gisou van der Goot[1] and Bruno Lemaitre[1].

[1] Ecole Polytechnique Fédérale de Lausanne, School of Life Sciences, Global Health Institute, Station 15, Lausanne, Switzerland , [2] Centre de Génétique Moléculaire, CNRS, 91198 Gif-sur-Yvette, France, [3] Structural Immunology, AFMB, CNRS-UMR 6098 Campus de Luminy, Marseille France

Address for correspondence: onya.opota@epfl.ch

Pseudomonas entomophila is a recently identified bacterium that is able to infect and kill *Drosophila* as well as insects from different orders. Ingestion of *P. entomophila* inflicts severe damage to the *Drosophila* intestine. How damage is inflicted remains however unknown. A screen for virulence factors led to the identification of a secreted protein that plays an important role in the damage inflicted by *P. entomophila* to the *Drosophila* gut. We showed that this protein is a pore-forming toxin (PFT) that we named Monalysin. We expressed and purified Monalysin and demonstrated its ability to form pores in lipid bilayers and to induce hemolysis. Electron microscopy reveals that Monalysin oligomerizes into ring-like structures that insert pores into the plasma membrane of target cells leading to the disruption of membrane impermeability and cell death. *In vivo*, a *Pseudomonas entomophila* strain deficient of *monalysin* displayed reduced cytotoxicity and lethality towards *Drosophila*. A study of Monalysin regulation revealed that its synthesis is under the control of both the two component system GacS/GacA and the Pvf (Pseudomonas Virulence Factor), a secondary metabolite synthesized by a non-ribosomal peptide synthetase (NRPS). We also found that Monalysin is processed by proteolytic cleavage. Interestingly, this cleavage is due to an endogenous protease secreted by *P. entomophila*. Here, we will describe the properties of this new pore-forming toxin and its contribution to *P. entomophila* virulence towards insects.

Poster Papers Wednesday, 16:30-18:30

Fungi

Poster / Fungi, Wednesday 16:00 **F1 STU**

How complex is the *Metarhizium* community in an agricultural field?

Bernhardt M. Steinwender[1]; Jürg Enkerli[2]; Franco Widmer[2]; Jørgen Eilenberg[1]; Nicolai V. Meyling[1]

[1]Department of Agriculture and Ecology, Faculty of Life Sciences, University of Copenhagen, Thorvaldsensvej 40, DK 1871 Frederiksberg C., Denmark, [2]Agroscope Reckenholz-Tänikon Research Station ART, Reckenholzstrasse 191, 8046 Zürich, Switzerland

Address for correspondence: bmsw@life.ku.dk

The *Metarhizium anisopliae* lineage has recently been shown to comprise several cryptic species. The abundance and distribution of the resulting nine terminal taxa in different ecosystems is still largely unknown. Specifically, it is not clear which of the newly described species constitute the *Metarhizium* community in the soil of agricultural fields. *Metarhizium* spp. from a single agricultural field (~ 1 ha, divided into 32 plots) in Denmark were isolated, using *Tenebrio molitor* as bait insect. For assessment of the genetic diversity within the soil of this field 116 isolates were analyzed using DNA sequencing (5'- intron region of Elongation Factor 1-alpha) and simple sequence repeat (SSR) marker analysis. *M. brunneum* (86.3%), *M. robertsii* (11.3%) and *M. majus* (3.4%) where identified in the soil samples of the arable field revealing co-existence of these species within the same field. Several genotypes of each species were identified based on SSR markers revealing a complex *Metarhizium* community. Differences in abundance of the species and their genotypes in this field indicate that some fungal genotypes may be better adapted to the soil environment of an agricultural field than others.

Poster / Fungi, Wednesday 16:00 **F2**

Genetic diversity of multiple single-spore isolations of *Beauveria bassiana* from individual, naturally infected grasshoppers.

Stefan T. Jaronski and John A. Gaskin

USDA-Agricultural Research Service, Northern Plains Agricultural Research Laboratory, 1500 N. Central Ave., Sidney MT 59270 USA

Address for correspondence: stefan.jaronski@ars.usda.gov

As preliminary to a population genetics study of 300 *Beauveria bassiana* isolated from natural infections among grasshoppers in the Northern Plains of the U.S., we subjected 10-12 *B. bassiana* single spore isolates from each of 10 grasshoppers (N= 116 isolates) to genetic analysis via the 738 base pair sequence of the internal segment of the *Bloc* gene region, using primers B22U and B822L. We found a total of 4 haplotypes among the 10 grasshoppers. Nine of the 10 grasshoppers were monotypic for their *B. bassiana* haplotypes, but one grasshopper had two *B. bassiana* haplotypes, with a genetic distance between the two groups of 0.050 using a Generalized Time-Reversible (GTR) model of evolution. The four haplotype genetic distances varied from 0.050 down to 0.001. In comparison, Rehner et al. (2011. Mycologia. doi:10.3852/10-302) observed that their *B. bassiana* isolates, collected from a wide diversity of sources, had a maximum genetic distance of 0.786. Our isolates segregated as 66 haplotype 1 (5 grasshoppers); 2 haplotype 2 (1 grasshopper, which also contained 8 haplotype 1 isolates); 36 haplotype 3 (3 grasshoppers); and 12 haplotype 4 (1 grasshopper). Among the uniform *Bloc* haplotypes we observed both morphological differences and oosporein production differences, indicating more genetic diversity than indicated by the *Bloc* region. Higher resolution analyses are being conducted.

Poster / Fungi, Wednesday 16:00 **F3 STU**

Phylogenetic and pathogenic divergence within *Metarhizium majus* lineage

Oumi Nishi; Kazuhiro Iiyama; Chisa Yasunaga-Aoki; Susumu Shimizu

Laboratory of insect pathology and microbial control, Institute of biological control, Kyushu University, Hakozaki 6-10-1, Higashi-ku, Fukuoka, Japan

Address for correspondence: sshimizu@grt.kyushu-u.ac.jp

The genus *Metarhizium* is one of the most phylogenetically and pathologically studied genera of entomopathogenic fungi. However, the relation between phylogeny and pathogenicity is not well known. The understanding of the relation is important to clarify the mechanism of host specificity and the process of pathogenic adaptation of entomopathogenic fungi. Thus we did phylogenetic analysis and comparative virulence assay of *M. majus* isolated from *Protaetia orientalis* (Scarabaeidae, Cetoniinae) larvae and from soil in Japan. Phylogenetic analysis of the 5' end of translation elongation factor 1alpha gene showed that the Japanese *M. majus* isolates were divided into large two clades; One isolates from a *P. orientalis* larva and two from soil were close to Australian isolates from *Anoplognathus* sp. (Scarabaeidae, Rutelinae) and one isolates from soil was close to the ones from *Oryctes* spp. (Scarabaeidae, Dynastinae). Virulence assay toward *P. orientalis* revealed that only the former three isolates caused mycoses. Phylogenetic analysis of the intergenic spacer region of ribosomal DNA indicated the three isolates have distinct lineage from the other *M. majus* strains. These results indicated the various

pathogenic adaptations toward Scarabaeidae species might have happened within *M. majus* clade.

Poster / Fungi, Wednesday 16:00 **F4**

Biological control of *Rhipicephalus microplus*: An intensive search for promising fungal biological control agents

Éverton K. K. Fernandes[1,2,a], Isabele C. Angelo[1], Drauzio E. N. Rangel[2], Thiago C. Bahiense[1], Áurea M. L. Moraes[3], Donald W. Roberts[2] and Vânia R. E. P. Bittencourt[1]

[1]Curso de Pós Graduação em Ciências Veterinárias, Universidade Federal Rural do Rio de Janeiro, Seropédica, RJ, Brazil; [2]Department of Biology, Utah State University, Logan, UT, USA; [3]Laboratório de Taxonomia, Bioquímica e Bioprospecção de Fungos, Instituto Oswaldo Cruz, Fundação Oswaldo Cruz, Rio de Janeiro, RJ, Brazil; [a]Current address: Instituto de Patologia Tropical e Saúde Pública, Universidade Federal de Goiás, Goiânia, GO, Brazil.

Address for correspondence: evertonkort@iptsp.ufg.br

The current study evaluates the virulence of several fungal isolates to larvae of the cattle tick, *Rhipicephalus microplus*, in the laboratory as part of an effort to identify isolates with promise for effective biocontrol of *R. microplus* in the field. Sixty fungal isolates, encompassing 5 *Beauveria* spp. and 1 *Engyodontium albus*, were included in this study. In addition to bioassays, the isolates were characterized morphologically and investigated as to their potential for conidial mass production. These findings were correlated with previous reports on the same fungal isolates that discuss their natural UV-B tolerances, thermotolerances, cold activities, and genotypes. Isolates CG 464, CG 500 and CG 206 were among the most virulent *Beauveria* isolates tested in this study. All fungal isolates presented morphological features consistent with their species descriptions. Of the 53 *B. bassiana* isolates, five (CG 481, CG 484, CG 206, CG 235 and CG 487) had characteristics that qualified them as promising biological control agents of *R. microplus*, viz., mean LC_{50} between 10^7 and 10^8 conidia ml^{-1}; produced 5,000 conidia or more on 60 mm^2 surface area of PDAY medium; and, in comparison to untreated (control) conidia, had the best conidial tolerances to UV-B (7.04 kJ m^{-2}) and heat (45 °C, 2 h) of 50% or higher, and conidial cold (5 °C, 15 d) activity (mycelial growth) higher than 60%. The current study of 60 *Beauveria* spp. isolates, therefore, singles out a few (five) with high potential for controlling ticks under field conditions.

Poster / Fungi, Wednesday 16:00 **F5 STU**

Virulence and thermotolerance of acaropathogenic fungi for the control of the two-spotted spider mite, *Tetranychus urticae*

Tae Young Shin; Won Woo Lee; Seung Hyun Ko; Sung Min Bae; Jae Bang Choi; Soo Dong Woo

Department of Agricultural Biology, Chungbuk National University, Cheongju, 363-763, Korea

Address for correspondence: sdwoo@cbnu.ac.kr

The two-spotted spider mite, *Tetranychus urticae* Koch, is an economically important pest of crops of plant grown in the field or greenhouse worldwide. It has recently become a serious problem because of the continuous use of acaricides resulting in resistance among spider mite population. Thus, there is a need to find alternative control measures to suppress spider mite populations. In this study, we report the screening result of pathogenic fungi for the control of spider mite. Initial screenings were performed using 352 isolates of putative pathogenic fungi from Korea soils. As results, 11 strains of acaropathogenic fungi were isolated from 8 cadavers of spider mite supporting fungal conidiation. These isolated were identified as four isolates of *Beauveria bassiana* (6, 2R-3-3-1, 2R-4-5, 2R-4-7), two isolates of *Metarhizium anisopliae* (4-2, 2-2), one isolate of *Clonostachys rosea* 5-2, one isolate of *Lecanicillium attenuatum* 4-1, one isolate of *Pochonia suchlasporia* 2R-3-1, one isolate of *Aspergillus flavus* 7 and one isolate of *Isaria lilacinus* 2R-4-6 by microscopic examination and genetic sequencing of the ITS region. Based on the screening results, eleven isolates were tested for their virulence against adult spider mites and thermotolerance. All fungal isolates were pathogenic to spider mite but mortality and thermotolerance varied with isolates. These acaropathogenic fungi may be useful to develop eco-friendly acaricide to control two-spotted spider mite.

Poster / Fungi, Wednesday 16:00 **F6**

Effect of the alarm pheromone of the rice stink bug, *Oebalus pugnax,* on the *in vitro* germination and development of the entomopathogenic fungi *Beauveria bassiana and Metarhizium anisopliae.*

Maynard L. Milks; James R. Fuxa; Jason C. Hamm; Arthur R. Richter; and Michael J. Stout.

Department of Entomology, Louisiana State University, Baton Rouge, LA, USA 70803

Address for correspondence: mmilks1@lsu.edu

The rice stink bug, *Oebalus pugnax* (F), is one of the most injurious pests of rice, *Oryza sativa* (L), in the southern United States. This insect reduces rice yields, grain quality and introduces pathogenic fungi causing "pecky" rice for which growers are penalized. *O. pugnax* has traditionally been controlled with chemical insecticides but many of these compounds have since been or are pending removal from the marketplace because of human safety and environmental concerns. Entomopathogenic fungi such as *Beauveria bassiana* (Bals.-Criv) and *Metarhizium anisopliae* (Metsch.) offer a promising alternative for being developed as bioinsecticides against *O. pugnax.* These fungi often cause natural epizootics in their host target populations but do not infect vertebrates and non-target insects. In particular, *B. bassiana* has been reported to infect stink bugs but surprisingly has not been observed to cause natural epizootics in pentatomids. The high temperatures (30-35°C) commonly observed in North American rice fields is likely to be a key factor curtailing fungal outbreaks on *O. pugnax.* Stinkbugs, however, also produce defensive secretions when startled by a predator. These secretions often contain antimycotic aldehydes (e.g. hexanal) raising the possibility that they may also act as antifungal defenses. The purpose of this study is two-fold: First, we characterized the chemical components of the alarm pheromone of *O. pugnax.* Second, we investigated the aromatic effects of each component and of the reconstituted pheromone blend on the *in vitro* germination and vegetative growth of two isolates of *B. bassina* and *M. anisopliae*.

Poster / Fungi, Wednesday 16:00 **F7 STU**

The ability of *Aphidius colemani* to vector entomopathogenic fungi *Lecanicillium* spp. against insect *Aphis gossypii* (Bio-cooperated control for cotton aphids)

Yuuna Saitou[1]; Junya Tone[1]; Masanori Koike[1]; Daigo Aiuchi[2;]

[1]Department of Agro-environmental Science, Obihrio University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido 080-8555, Japan, [2]National Research Center for Protozoan Diseases, Obihrio University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido 080-8555, Japan.

Address for correspondence: aigo@obihiro.ac.jp

The ability of *Aphidius colemani* to vector *Lecanicillium* spp. conidia during host searching and/or oviposition behavior to a colony of uninfected *Aphis gossypii* was investigated. Experiments were conducted to release an *A. colemani* which treated with *Lecanicillium* spp. (1. direct inoculation with fungi; 2. picked up fungal conidia from contaminated leaf disk) to the screen cage containing potted cucumber infested with aphid. These two different experiments indicated the similar tendency in transition of aphid population. Aphid population were exponentially increased in control

plots during 10 days (791.2~819.0 aphid/plant). In contrast, aphid population did not increase in *A. colemani* released plots up to 8 days after parasitoid release and then rapidly increase in 10 days (130.1~216.3 aphid/plant). In parasitoid treated with *Lecanicillium* spp. Released plots, aphid density was maintained at low level up to 10 days after parasitoid release (60.6~66.5 aphid/plant). Experiments indicated that *A. colemani* has the ability to transmit fungal conidia to host insects. Furthermore, the number of mummy were same level on parasitoid and parasitoid + fungi plots, hence it suggested that *Lecanicillium* spp. were no noxious effect on the egg – laying performance of *A. colemani*. Considering *Lecanicillium* spp. could survive on leaf surface by using plant exudate, the ability of *A. colemani* to vector *Lecanicillium* spp. conidia which deposited on leaf surface to aphid colony might largely contribute to dispersal of *Lecanicillium* spp. In this study, it was revealed that *Lecanicillium* spp. acted additively to control the aphid by *A. colemani* vectoring as a parasitic vector.

Poster / Fungi, Wednesday 16:00 **F8**

Biological evaluation of *Cordyceps militaris*

InPyo Hong[1]; SoonOk Woo[1]; SangMi Han[1]; MiKyeong Lee[2]; Richard A. Humber[3]
[1]National Academy of Agricultural Science (NAAS), Suwon, Rep. of Korea, [2]College of Pharmacy & CBITRIC, Chungbuk National University, Cheongju, Rep. of Korea, [3]USDA-ARS Biological IPM Research, Ithaca, NY, United States.
Address for correspondence: iphong20@korea.kr

There are more than 80 species of entomopathogenic fungi, of which about 10 species have been reported to produce a fruiting body in Korea. Fruiting bodies of *Cordyceps militaris* have been widely used and valued in traditional herbal market. This study seeks the biological and pharmaceutical value by *C. militaris* on obesity. Obesity is no longer considered to be only a cosmetic problem but associated with lots of pathological disorders, including diabetes, hypertension, atherosclerosis and cancer. The total ethanol extract of *C. militaris* showed significant inhibitory activity on adipocyte differentiation. Adipocyte differentiation was assessed employing 3T3-L1 preadipocyte cell line as an in vitro assay system by measuring the fat accumulation using Oil Red O staining. Activity guided fractionation using various column chromatographic method leds to the isolation of cordycepin, guanosine and tryptophan. All these three compounds showed significant inhibitory activity on adipocyte differentiation. Further studies with interval treatment such as 0-2, 2-4 and 4-8 days differentiation induction suggested that guanosine acts on early stage whereas cordycepin acts on middle stage. Tryptophan exerted inhibitory activity when treated early or middle stage of differentiation. Taken together, *C. militaris* and its constituents might be useful in the prevention of obesity.

Poster / Fungi, Wednesday 16:00 **F9 STU**

Evaluation of entomopathogenic fungus *Lecanicillium muscarium* hybrid strain 2aF43 formulation as biological control agent of greenhouse whitefly, *Trialeurodes vaporariorum*

Hiroto Shinomiya[1, 4], Daigo Aiuchi[2], Willem J. Ravensberg[3], Masanori Koike[1*]
[1]Depertment of Agro-enviromental Science, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido 080-8555, Japan , [2]National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido 080-8555, Japan, [3]Koppert Biological Systems, Veilingweg 17, 2650 AD Berkel en Rodenrijs, the Netherlands, [4]Present address: Department of Bioregulation and Biointeraction, Graduate School of Agriculture, Tokyo University of Agriculture and Technology, Fuchu, Tokyo 183-8509, Japan
Address for correspondence: koike@obihiro.ac.jp

The *Lecanicillium muscarium* hybrid strain 2aF43 was obtained by protoplast fusion and shown to have high control potential of greenhouse whitefly at *in vitro bioassay*, and Mycotal-formulation is commercial biocontrol agent having high virulence against whitefly. In this study, 2aF43 was formulated in a similar method of Mycotal by Koppert Biological Systems, then we investigated the control effects of these two formulations (average conidial concentrations of 2aF43-formulation and Mycotal-formulation were 1.7×10^4 spore/ml and 3.0×10^6 spore/ml, respectively) against greenhouse whitefly on tomato plants in greenhouse. In control plots, the number of adult whitefly significantly increased up to 354 adults/ leaf during 8 weeks from the first spraying day. Whereas, in 2aF43-formulation and Mycotal-formulation plots, adult density were remained constantly low level (0.6 to 15.9 adults/leaf and 0.5 to 11.3 adults/ leaf, respectively) for 7 weeks. The density of 2aF43 propagules on sprayed tomato leaves was 3.7×10^3 cfu/ cm^2, indicating possibility that not only persisting, this strain also growing on leaves under given conditions. Additionally, the density of 2aF43 propagules on no-sprayed tomato leaves was 69.0 cfu/ cm^2, indicating possibility that fungal propagules of this strain were transmitted from sprayed tomato leaves to no-sprayed tomato leaves in some way (e.g., insect movement, wind, etc.). Evidences suggested that although concentration of hybrid strain 2aF43-formulation was substantially lower than Mycotal-formulation, it still had the potential for controlling early emergence of greenhouse whitefly and the possibility for long term effect in greenhouse use.

Poster / Fungi, Wednesday 16:00 **F10**

UV-B and Heat induced Post-stress growth delay

Chad A. Keyser[1], Everton K. K. Fernandes[1, 2], Donald W. Roberts[1]
[1] *Department of Biology, Utah State University, Logan, UT 84322-5305, USA*
[2]Current address: Instituto de Patologia Tropical e Saúde Pública, Universidade Federal de Goiás, Goiânia, GO, Brasil 74605-050
Address for correspondence: donald.roberts@usu.edu

Isolates of the entomopathogenic fungus *Metarhizium* spp. vary in their tolerances to two very important field stressors: high temperature and solar radiation. Laboratory tests designed to evaluate isolates' tolerance to environmental stress generally measure spore mortality after a single high-level exposure to that stress. We report here on the effects of sub-lethal dosages of heat and UV-B irradiation on several *Metarhizium* species and isolates. Conidia of 17 *Metarhizium* isolates were exposed to 45ºC heat for 5 hours, 17 other isolates were exposed to UV-B radiation for 3 hours. With both treatments percent germination was assessed at 400x after 24 and 48 hours incubation. Additionally, an in-depth study was conducted with eight isolates of *Metarhizium* (three *M. acridum*, two *M. guizhouense*, two *M. robertsii*, and one *M. brunneum*) in which conidia were exposed to sub-lethal doses of UV-B (2h) radiation and 45ºC heat (4h); after which, percent germination was assessed at 6, 12, 24, 36, and 48 hours and compared to untreated controls. A stress-induced delay in germination and/or growth [designated here as post-stress growth delay (PSGD)] was commonly noted with both heat and UV-B sub-lethal exposure. Of the 17 isolates exposed to **heat** for 5 hours, all but one showed extreme PSGD; the isolate with the least heat induced PSGD was ARSEF 324, a *M. acridum* isolate known to have high tolerance to heat. Of the17 isolates exposed to **UV-B** for 3 hours, all showed some level of PSGD; the isolate with the fastest recovery was DWR 1280, a *M. robertsii* isolate from Idaho, USA. In the more in-depth study, some degree of PSGD was observed in all the isolates after exposure to UV-B radiation or high heat. ARSEF 324 exhibited a significant heat-induced delay only at 12 hour after treatment. DWR 346 had the lowest UV-B induced PSGD of the isolates tested. Delayed germination due to repeated (daily) environmental stresses may serve to seriously impede fungal metabolism and, thereby, render an isolate

ineffective as a biological control agent. Accordingly, when selecting isolates for field use their performance in relation to PSGD should be given serious consideration.

Poster / Fungi, Wednesday 16:00 **F11 STU**

Cold activity and resistance of the entomopathogenic fungus *Tolypocladium spp* to UV-B radiation and heat

Maiara P. Santos, Luciana P. Dias, Michele S. Pinheiro, Paulo C. Ferreira, Drauzio E. N. Rangel
Instituto de Pesquisa e Desenvolvimento, Universidade do Vale do Paraíba, São José dos Campos, SP, 12244-000 Brazil
Address for correspondence: drauzio@pq.cnpq.br

Studies on the stress resistance of insect-pathogenic fungi are very important to better understand the survival of these organisms in the environment. Therefore, this study examined the cold activity (8 ± 1 °C for seven days), UV-B tolerance (weighted UV-B irradiance at 870.44 mW m^{-2} for 1, 2, 3 and 4 h) and wet-heat tolerance (45° C for 1, 2, 3 and 4 h) of two isolates of *Tolypocladium cylindrosporum* (ARSEF 3392 and 5558), one isolate of *T. geodes* (ARSEF 3275), and two isolates of *T. Inflatum* (ARSEF 4772 and 4877) based on their germination, compared with a *Metarhizium robertsii* isolate. After 3 h of UV-B exposure, the two isolates of *T. cylindrosporum* were more resistant than the other *Tolypocladium* isolates but were similar to the *M. robertsii* isolate. All *Tolypocladium* isolates, however, were less tolerant to UV-B radiation than the isolate of *M. robertsii* after 4 h of UV exposure. The isolates of *T. inflatum* and *T. geodes*, which had similar heat tolerance, were the least heat tolerant compared with the isolates of *T. cylindrosporum* and *M. robertsii*. After 4 h of heat exposure, the germination of *T. inflatum* and *T. geodes* was approximately 10%. The heat tolerance of the *T. cylindrosporum* isolates was similar to the *M. robertsii* isolate. For cold activity, both *T. cylindrosporum* isolates germinated to ca. 100% in only three days, both *T. inflatum* isolates germinated approximately 50% after three days. *T. geodes* germinated approximately 30% after five days and on the sixth day it germinated approximately 80%. The *M. robertsii* isolate did not germinate after seven days. The isolates of *T. cylindrosporum*, therefore, were the most heat and UV-B tolerant as well as had the highest cold activity as compared with the other species. The tolerance of *M. robertsii* to UV-B radiation and heat was similar to the tolerance of *T. cylindrosporum*.

Poster / Fungi, Wednesday 16:00 **F12**

The effect of aphid cuticular waxes and pigment on the infection process of *Metarhizium anisopliae*

Bree A Wilson, Ben J Stodart, Caroline I Hauxwell, Gavin J Ash
EH Graham Centre for Agricultural Innovation (an alliance between Charles Sturt University and Industry & Investment NSW). Locked Bag 588, Wagga Wagga, NSW, 2678, Australia
Address for correspondence: brwilson@csu.edu.au

Sucking insects like aphids can cause significant yield losses in agriculture due to the direct effects of feeding and the indirect effects associated with the spread of viruses. In the current project, *Metarhizium anisopliae* isolate EFD 251 (Australia) has been shown to have insecticidal activity against a range of crop pests including aphids, mirids, green vegetable bug, thrips, Rutherglen bug and *Helicoverpa*. Several species of aphid transmit viruses in grain crops in Australia and include the waxy cabbage aphid *Brevicoryne brassicae*, the dark pigmented oat/wheat aphid *Rhopalosiphum padi* and the light pigmented rose-grain aphid *Metopolophium dirhodum*. In this study, we present a time-course of the infection process and blastospore formation by *M. anisopliae* on several aphid species. Using light, scanning and transmission microscopy we investigate the role of the wax layer and the colour of the cuticle on 1) the adhesion and germination of conidia, 2) mycelium production and 3) penetration of germ tubes through the aphid cuticle. The results gained from this study, together with information obtained from complementary *M. anisopliae* pathogenicity trials, will yield important information about the surface chemistry of aphids and how this may influence adhesion of conidia and, therefore, the ultimate effectiveness of fungal-based biopesticides.

Poster / Fungi, Wednesday 16:00 **F13 STU**

Evaluation of infectivity and pathogenicity of anamorphic entomopathogenic fungi isolated from wild mosquitoes in Japan and Burkina Faso against adult female *Anopheles stephensi*

Minehiro Ishii[1];Mitsugu Ishiyama[1]; Masanori Koike[1];Shinya Fukumoto[2];Hirotaka Kanuka[2]; Junya Takeshita[1];Daigo Aiuchi[2]
[1]Department of Agro-environmental Science, Obihiro University of Agriculture &Veterinary Medicine, Obihiro, Hokkaido 080-8555, Japan, [2]National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine; Obihiro, Hokkaido 080-8555, Japan.
Address for correspondence: aigo@obihiro.ac.jp

Mosquito vector control is an important part of controlling malaria. Although the use of chemical insecticides is the mainstay of malaria vector control, development of insecticide resistance have been reported. Now new vector control approaches are needed. So, the aim of this study was to search promising isolates as fungal biopesticide of malaria vector mosquito. Exogenous and endogenous anamorphic entomopathogenic fungi were isolated from whole body of wild adult mosquitoes collected in Japan and Burkina Faso, and their infectivity and pathogenicity were assessed. Only 13 out of 37 isolates showed infectivity against female *Anopheles stephensi*; Of the total, 4 *Lecanicillium* spp, 7 *Beauveria bassiana*, 1 *Isaria farinosa* and 1 *Paecilomyces carneus*. This low infectivity might be due to these candidate of fungal isolates including not only endogenous fungi but exogenous one. Among them, 3 isolates of *Lecanicillium* spp and 3 isolates of *Beauveria bassiana* which showed higher infectivity were applied for bioassay of pathogenicity. As a result, 2 isolate of *Lecanicillium* spp and 3 isolates of *Beauveria bassiana* significantly reduced survival period of *An. stephensi* compared with control ($p < 0.01$). The median lethal time of fungal treated adults were ranged from 6.7 days to 13.7 days, while, control plot was 15.2 days. These results revealed that some anamorphic entomopathogenic fungi isolated from wild mosquito might have potential for controlling vector mosquito. Furthermore, we have been continued for evaluation of more than 65 isolates of endogenous entomopathogenic fungi. We will also discuss about infectivity/pathogenicity of these isolates.

Poster / Fungi, Wednesday 16:00 **F14 STU**

Isolation of anamorphic entomopathogenic fungi from wild mosquitoes and evaluation of their latent infection

Junya Takeshita[1]; Mitsugu Ishiyama[1]; Minehiro Ishii[1]; Masanori Koike[1]; Shinya Fukumoto[2]; Hirotaka Kanuka[2]; Daigo Aiuchi[2]
[1]Department of Agro-environmental Science, Obihrio University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido 080-8555, Japan, [2]National Research Center for Protozoan Diseases, Obihrio University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido 080-8555, Japan.
Address for correspondence: aigo@obihiro.ac.jp

In this study, entomopathogenic fungi were isolated from wild adult mosquitoes to understand natural state of relationship between mosquitoes and entomopathogenic fungi and to construct fungal culture library specialized for anamorphic entomopathogenic fungi originated from mosquitoes. Initially, entomopathogenic fungi were isolated from wild mosquitoes

collected in Japan and Burkina Faso and molecular-based identification was conducted. As a result, 78 *Beauveria bassiana*, 4 *B. brongniartii*, 29 *Isaria farinosa*, 4 *Paecilomyces carneus*, 1 *P. lilacinus* 14 *Lecanicillium araneicola*, 94 *Lecanicillium* spp., 8 *Simplicillium lamelicola* and 1 *S. lanosoniveum* were obtained from Japanese mosquitoes, and 3 *B. bassiana*, 1 *I. farinosa*, 77 *L. araneicola* and 13 *S. lanosoniveum* were obtained from Burkina Faso mosquitoes. Secondly, entomopathogenic fungi were isolated from inside of the mosquito body by sterilizing their integument. Consequently, 41 *B. bassiana*, 1 *I. farinosa*, 1 *I. fumosorosea* and 22 *Lecanicillium* spp. were detected, and the latent infection rate of active mosquito (they were collected by hand-net catches) was 4.65%. Moreover, concomitant infection by *B. bassiana* and *Lecanicillium* sp. was demonstrated. Finally, entomopathogenic fungi were isolated from supernatant liquid of paddle where was breeding site of mosquitoes. Consequently, *B. bassiana* and *Lecanicillium* spp., *Isaria* spp. were isolated from these samples. It is highly possible that mosquitoes picked up entomopathogenic fungi during oviposition or emergence at such hydrosphere. These result indicated that various anamorphic entomopathogenic fungi correlate with wild mosquitoes by infecting inside body and/or adhering to integument. In this study, fungal culture library housed 392 isolates of anamorphic entomopathogenic fungi was constructed.

Poster Papers Wednesday, 16:30-18:30

Microsporidia

Poster / Microsporidia, Wednesday 16:00 **MS1 STU**

The effects of two microsporidian pathogens on the two-spotted ladybeetle, *Adalia bipunctata* L. (Coleoptera: Coccinellidae)

Thomas Steele; Susan Bjørnson
Biology Department, Saint Mary's University, 923 Robie Street, Halifax, NS B3H 3C3 Canada.
Address for correspondence: steelem4@hotmail.com

The two-spotted lady beetle, *Adalia bipunctata*, and the convergent lady beetle, *Hippodamia convergens*, are available for biological pest control in North America. These beetles are hosts to microsporidian pathogens, which can be inadvertently imported and released when beetles are used in biological control programs. Although multiple microsporidian pathogens have been found within a single host, little is known about the effects of multiple microsporidian infections. In this study, egg cannibalism was used to examine horizontal transmission of *Tubulinosema hippodamiae* from *H. convergens* and an undescribed microsporidium from *A. bipunctata* (alone and in combination) in *A. bipunctata* larvae. Effects on host fitness (larval development, larval mortality, sex ratio, fecundity and adult longevity) were also examined. Spores were detected in the majority of smear preparations of individuals which were fed microsporidia-infected eggs and molecular analysis confirmed the identity of both pathogens. Development was significantly longer for larvae that were infected with the unidentified microsporidium than the uninfected and *T. hippodamiae*-infected larvae. No significant difference was found between larvae infected with the unidentified microsporidium and larvae infected with both microsporidia. This suggests that *T. hippodamiae* had no effect on larval development and no conclusions can be made regarding the effects of multiple pathogens on larval development. Mortality was significantly higher in *T. hippodamiae* infected larvae than those infected with the unidentified microsporidium. The toxic species-specific alkaloids that coat coccinellid eggs could explain high mortality rates. Sex ratios of emerged adults were about 1:1 (♀:♂) and no significant difference was observed between infected and uninfected beetles. The pathogens had no effect on adult fecundity and longevity. Pathogen transmission was studied under optimal conditions during this study. Microsporidia may have a greater impact on host fitness under natural conditions. This study still raises concerns regarding the use of imported lady beetles for biological control.

Poster / Microsporidia, Wednesday 16:00 **MS2**

Prevalence of *Nosema* disease in honeybee in Korea

NamSuk Kim[1]; YongSoo Choi[1]; InPyo Hong[1]; Richard A. Humber[2]
[1]National Academy of Agricultural Science (NAAS), Suwon, Rep. of Korea, [2]USDA-ARS Biological IPM Research, Ithaca, NY, United States.
Address for correspondence: iphong20@korea.kr

Korea has 34,102 beekeepers with 1,858,574 hives including Western honeybee (*Apis mellifera*) and Oriental honeybee (*Apis cerana*). Nosema disease is one of the most serious diseases in honeybees causing significant production losses in Korea. The aim of the present study was to determine the prevalence of *Nosema apis* in Korea. Worker honeybees (*Apis mellifera*) were sampled in 13 provinces. Collections were made from 22 apiaries in the flowering season of *Robinia pseudoacacia* and 13 apiaries in non blossom 2009. Nosema infection was encountered in 73% of the apiaries sampled and the average Nosema spore per worker was 3.1 million in the flowering season. Infection rate of Nosema was 85% of the apiaries and the average Nosema spore per worker was 3.6 million in no blossom season. The flowering season showed a lower Nosema infection rate and spore count than non blossom season. Also the highest level of infection was observed to be in spring and autumn among annual survey.

Poster / Microsporidia, Wednesday 16:00 **MS3**

Agents effective against the germination of *Nosema ceranae* spores

Martin A. Matisoff; Thomas C. Webster
Land Grant Program, Kentucky State University, Frankfort KY, 40601 USA
Address for correspondence: Martin.matisoff@kysu.edu

Nosema ceranae is an obligate, intracellular microsporidium infecting the honey bee (*Apis mellifera*) midgut. It is serious and widespread in much of the world. One control strategy is to eliminate spores from stored beekeeping equipment or to destroy their ability to germinate. Our approach was to consider how spores are affected by long periods on beekeeping equipment and how stimuli in the honey bee midgut cause the spores to germinate. Our long term goal is to use our data to develop safe, effective, and inexpensive methods for sustainable control of *N. ceranae*. Possibly, vacuum treatment in a desiccator simulates a long period of inactivity on equipment. In addition, sugars and salts in food ingested by the bee may contribute to stimuli for spore germination. We applied a concentrated suspension of *N. ceranae* spores to microscope slides, allowed them to air dry, and then put the slides into an evacuated desiccator for one, two or four hours. After the vacuum treatment, the spores were treated with various dilutions of trehalose and buffer made of $NaHCO_3$ and NaCl and then examined under phase contrast at 400x. The vacuum treatments and the sugar/buffer media caused many of spores to appear dark. However, a large fraction of the dark spores did not have polar filaments associated with them. Generally, spores turn dark when observed by phase contrast as they germinate and release their polar filaments. Polar filaments are normally easy to see, especially after the slide has air-dried with the cover glass in place. Consequently, we suspect that many of the spores have been affected in some way by these agents, thereby preventing spores from releasing their filaments. Further work will determine whether spores treated in this way can infect bees.

Poster / Microsporidia, Wednesday 16:00 **MS4**

***Nosema ceranae* in migratory beekeeping in the United States**

Thomas C. Webster[1]; James D. Elllis[2]; Melissa L. Calhoun[1]; Kirk Pomper[1]; Kyle Schneider[1]

[1] Land Grant Program, Kentucky State University, Frankfort KY, 40601 USA

[2] Department of Entomology and Nematology, University of Florida, Gainesville, FL, USA

Address for correspondence: Thomas.webster@kysu.edu

The Microsporidian *Nosema ceranae* has been implicated in widespread honey bee (*Apis mellifera*) colony mortality and is now endemic in live colonies in the United States and some other countries. Infections are probably worse when the colonies are stressed by parasites and other pathogens. Possibly long distance moving of hives by migratory beekeepers in the U.S. is an additional factor in causing outbreaks of this pathogen. Many beekeepers routinely move their hives from Florida to California and back, a round trip distance of about 10,000 km, to pollinate the almond orchards every February and March. In this trip, the bees are confined for several days, and must then orient to their new location. Often, little forage other than almond nectar and pollen is available in the almond orchards. We sampled bees from 20 hives in each of three beekeeping operations, before and after their hives made this long trip. *N. ceranae* was detected by polymerase chain reaction. We found that *N. ceranae* infections decreased in two of the operations, from 85% of hives infected to 70%, and from 55% to 18%. In the other operation, the infections increased from 70% to 85%. Possibly, these results are related to co-infections with other pathogens and with the parasitic mites *Varroa destructor* and *Acarapis woodi*.

Poster / Microsporidia, Wednesday 16:00 **MS5**

Microsporidian pathogens in biological control agents of hemlock woolly adelgid

Leellen F. Solter[1], Wei-Fone Huang[1] and Bradley Onken[2]

[1] Illinois Natural History Survey, University of Illinois, 1816 S. Oak St., Champaign, IL 61820, USA; [2] USDA Forest Service, 180 Canfield Street, Morgantown, WV 26505 USA;

Address for correspondence: lsolter@illinois.edu

Latent or cryptic pathogens such as submicroscopic viruses and microsporidia may go undetected in quarantined and laboratory reared invertebrate hosts collected for biological control programs. Although most pathogens persist at low enzootic levels in field populations of their hosts, the laboratory setting provides a highly favorable environment for epizootics, and disease can quickly compromise expensive, long-term efforts to mass-rear and establish biological control agents. At least five microsporidian species have been recovered from four species of coleopteran predators of *Adelges tsugae* Annand, the hemlock woolly adelgid (HWA), the Asian imports *Sasajiscymnus tsugae* (Sasaji & McClure) and *Scymnus sinuanodulus* Yu & Yao, and *Laricobius nigrinus* Fender and *Scymnus coniferarum* Crotch collected in western U.S. Three beetle colonies infected with different species of microsporidia were compromised by the pathogens. Regular screening for one microsporidian species in a laboratory colony of *S. tsugae* showed an increase in prevalence from 12% to 50% in one rearing season, and high winter mortality was recorded among infected beetles in a preliminary semi-field experiment. This *Tubilinosema* sp. was infective to *L. nigrinus, S. sinuanodulus* and *Laricobius osakensis* Montgomery & Shiyake in laboratory host specificity testing. Predatory beetle species being collected or reared for release to control HWA are relatively host specific to hemlock and pine adelgids, increasing the risk that infected beetles will inoculate the feeding niche of the adelgids, thereby exposing conspecific individuals and other predatory species to the pathogens. Current studies will determine if the microsporidia from released mass reared hosts persist in the field.

Poster Papers Wednesday, 16:30-18:30

Nematodes

Poster / Nematodes, Wednesday 16:00 **N1 STU**

An investigation on efficacy of entomopathogenic nematodes on leopard moth, *Zeuzera pyrina* L. (Lep.: Cossidae) in Iran

Mahbobeh Ashtari[1], Mohammadreza Rezapanah[2], Javad Karimi[3]

[1]Universirt of Arak, Arak, Iran

[2]Biological Control Dept., Iranian Research Institute of Palnt Protection, Tehran, Iran

[3]Department of Plant Protection, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran

Address for correspondence: jkb@um.ac.ir

The leopard moth, *Zeuzera pyrina* (Lep. Cossidae) is a destructive wood borer in most parts of Iran. It poses unique management challenges because its immature stages live in cryptic, often inaccessible, habitats within host trees. Critical issue about chemical control of this pest as well necessary to find safe and environmental-friendly manners encouraged us to look for potential of some entomopathogenic nematodes (EPNs) against this caterpillar. Application of EPNs is a global trend in this decade, so efficiency of native and non-native EPNs, *Heterorhabditis bacteriophora* and *Steinernema carpocpase* were evaluated via laboratory assays on different larval stages of this insect. Also susceptibility of the *Z. pyrina* larvae to *H. bacteriophora* and *S. carpocpase* was evaluated in a field experiments. Both strains were used via injecting nematodes suspensions, 2000 IJs/larva in a bored gallery based on a CRD experiment which compared with control. The results indicated a high efficiency of *S. carpocapsae* than *H. bacteriophora* on larvae of *Z. pyrina*. All the larvae in bored galleries dead by *S. carpocapsae* under plastic cover and the mortality in treatment without cover were estimated 63%. The effect of trunk covering after application increased the efficacy to about 93% for *S.carpocapsae*. Comparison of mortality caused by *S. carpocapsae* versus *H.bacteriophora* demonstrated higher levels of mortality due to *S. carpocapsae.* So *S. carpocapsae* is an effective nematode species against larvae of *Z. pyrina* in walnut orchards. Results suggested that EPNs were more effective against *Z. pyrina* in moist heartwood habitats compared with species with larvae living in drier wood galleries. This is the first records about potential assay of native EPNs against *Z. pyrina* in Iran.

Poster / Nematodes, Wednesday 16:00 **N2STU**

A new isolate of the entomopathogenic nematode, *Steinernema* sp. (Nematoda: Steinernematidae), from Taiwan

Ching-Tzu Tseng; Roger F. Hou; Li-Cheng Tang

Department of Entomology, National Chung Hsing University, 250 Kuo Kuang Rd., Taichung, Taiwan.

Address for correspondence: lctang@dragon.nchu.edu.tw

A new nematode isolate no. 39 of *Steinernema* was collected from Pingtung County in southern Taiwan. Its infective juvenile, measuring 934±101 μm in length, is longer than *Steinernema abbasi* Taiwan strain, which is ca. 552 μm. Phylogenetic and similarity analyses based on rDNA internal transcribed spacer region sequences demonstrated that this isolate is close to *Steinernema guangdongenses* strain GDc339 of southern China but distinct from *S. abbasi*, and is an unrecorded one in Taiwan. In addition, our laboratory bioassays showed that *Steinernema* sp. no. 39 was highly pathogenic to the common cutworm, *Spodoptera litura*, and the greater wax moth, *Galleria mellonella*.

Poster / Nematodes, Wednesday 16:00 **N3**

Factors affecting hatching pattern of the eggs of *Strelkovimermis spiculatus* (Nematoda: Mermithidae)

María V. Micieli; Paula Risso; María F. Achinelly; M. de los Milagros Villar; Juliana Tarquini
Centro de Estudios Parasitológicos y de Vectores, CEPAVE (CONICET- CCT La Plata -UNLP), calle 2 Nº 584, (1900) La Plata, Buenos Aires, Argentina
Address for correspondence: vmicieli@fcnnym.unlp.edu.ar

We assessed the number of *Strelkovimermis spiculatus* Poinar and Camino, 1986 preparasites obtained from an initial known number of nematode eggs and how some abiotic factors (temperature-photoperiod, flooding-diseccation) could affect the number of emerged preparasites. Two groups of eggs, one maintained flooded and other under different number of flooding-diseccation events (each 15, 30 and 60 days), were performed. Each group of eggs was also studied under two conditions: 25º C and 14:12 (L:D) photoperiod, and at 16º C and 12:12 (L:D) photoperiod. When eggs were maintained flooded a higher total percentage of *S. spiculatus* preparasites was reported compared to treatments subject to drying-flooding cycles. The maximun percent of J2s emerged was found in the treatments maintained flooded at 16º C and 12:12 (L:D) photoperiod (30 ± 15.04 %). Preparasites were observed from 7 (25º C) and 14 (16º C) days suggesting this period as the minimun time for embryonic development. The period of time over which preparasites coming from the same batch of eggs were observed in flooded assays (98 and 112 days at 25ºC and 16ºC, respectively) suggested a nonsychronous hatching of nematode eggs possibly due to non uniform embryonation of eggs. The different periods of exposure of the assays to drought conditions no affected significantly the total average percentage of J2s obtained at 25º C and 14:12 (L:D) photoperiod, although at 16 ºC floods every 15 days showed the highest percent of emerged J2s. A schedule of flooding to optimize the mass rearing method for *S. spiculatus* is proposed

Poster / Nematodes, Wednesday 16:00 **N4**

Pathogenicity, Biology and Production of a new isolate of *Heterorhabditis bacteriophora* (Poinar, 1976) (Nematoda: Heterorhabditidae) from Argentina

María F. Achinelly; Daiana Eliceche; Nora Camino
Centro de Estudios Parasitológicos y de Vectores, CEPAVE (CONICET- CCT La Plata -UNLP), calle 2 Nº 584, (1900) La Plata, Buenos Aires, Argentina
Address for correspondence: fachinelly@cepave.edu.ar

Entomopathogenic nematodes belonging to the Heterorhabditidae are lethal parasites to soil-dwelling insects. They are commonly used as biological control agents of insects in cryptic habitats. Here, we provide data from the first evaluation of the pathogenicity of a native nematode, *Heterorhabditis bacteriophora* strain VE, isolated from organic gardens, in Villa Elisa, Buenos Aires province, Argentina. Ten *Galleria mellonella* (L.) larvae were exposed to the nematode at 15:1IJs/larva in Petri dishes (10 cm diameter) at 25°C. Six replicas with six repetitions were performed. Daily three replicates were observed and dead insects were dissected to record the mortality, parasitism and the nematode stage. In the other replicas, parasitized cadavers were placed in White traps to the emergence of the IJs. These results demonstrated the pathogenicity of *H. bacteriophora* causing between 70%-90% mortality. The nematode killed the insects 48 hs (57.14%) to 72 hs (42.85%) after contact. The parasitic period lasted between 10 to 14 days from the moment IJs entered the insect to the time when new IJs were formed, registering the highest number (42.1%) on day 12^{th}. Emergence continued until 31-32 days. We observed two generations of nematodes, the first one with hermaphrodites (4-7 after the death of the larvae) and the second one with females and males (8-13 days). The number of IJs per host varied between 9430 and 498000. On the basis of these results, *H. bacteriophora* was selected for further evaluation under field conditions, to control *Lobiopa insularis* (Coleoptera), an important emerging pest of strawberry crops in Argentina.

Poster / Nematodes, Wednesday 16:00 **N5**

Efficacy of entomopathogenic nematodes against Japanese pine sawyer, *Monochamus alternates* (Coleoptera: Cerambycidae)

Hwang Bin Yu[1]; Domg Woon Lee[2]; Ho Yul Choo[1]
[1]Department of Applied Biology, College of Agriculture & Life Sciences, Gyeongsang National University, Jinju, Gyeongnam, 660-701, Korea, [2]Department of Applied Biology, Kyungpook National University, Sangju, 742-711, Korea
Address for correspondence: whitegrub@knu.ac.kr

Japanese pine sawyer, *Monochamus alternatus* is an important vector of pine wood nematode, *Bursaphelenchus xylophilus*. Main control strategy in Korea was using pesticides although chemicals gave rise to many environmental and health hazards. The entomopathogenic nematode is one of alternative control agents against *M. alternates*. In the bait method using the great wax moth larvae in Japanese black pine, *Pinus thunbergii* log, *Steinernema carpocapsae* GSN1 and *Heterorhabditis* sp. Gyeongsan strains were highly effective against *Galleria mellonela* larvae at 2.5 cm deep of *P. thunbergii* log. There was no difference in the pathogenicity of *S. carpocapsae* GSN1 strain depending on exit hole depth of *Pinus thunbergii* log. In addition, *S. carpocapsae* GSN1 strain showed high pathogenicity against larvae of *M. alternates* (93% mortality at the rate of 80 Ijs) in petri dish and adult (55% mortality at the rate of 4000 Ijs) in pot. Effectiveness of *S. carpocapsae* GSN1 strain against larvae of *M. alternatus* beneath bark was higher than those in the wood of *P. thunbergii*. When *S. carpocapsae* GSN1 strain and selected synergistic pesticide, clothianidin were mixed and applied against *M. alternates* larvae in the log of *P. thunbergii* by spraying and soaking methods, soaking method was more effective than spraying method.

Poster / Nematodes, Wednesday 16:00 **N6**

Biological responses of *Rhynchophorous ferrugineus* to *Steirnenema carpocapase:* an example of a model system

Barbara Manachini, Vincenzo Arizza
Dep. Environmental biology and Biodiversity, University of Palermo, Via Archirafi 18, 90123 Palermo. Italy
Addresses for correspondence: barbara.manachini@unipa.it; b.manachini@unipa.it

Rhynchophorus ferrugineus (Coleoptera: Curculionidae) known as the Red Palm Weevil (RPW) is becoming more and more of a problem in Italy, and especially in Sicily, where it is well adapted. The infestations are mainly in the urban areas, and for that reason, chemical control is not advisable. Data from literature show that entomopathogenic nematodes (EPN) control RPW quite successfully in the field. However, results coming from the laboratories are often in contrast with each other and no data are available on precise doses. In this context we studied the Median Lethal Dose (DL50) and the Median Lethal Time (TL50) of young and older larvae and also of adults. The doses for the adults were twice that of the ones for larvae. Moreover, we investigate the effects of EPN on the immune system of RPW larvae. After a few hours, the nematodes were measured in the hemolymph of the insect and it appeared that the immune system was not activated by the presence of these foreign bodies. The nematodes suddenly mutated in the hemolymph totally undisturbed by the hemocytes. After 24 hours, the number of the hemocytes (THC) recorded in the larvae treated with *Steirnenema carpocapsae* were dramatically inferior compared to the THC found in the control larvae. EPN had

also a detrimental effect on the weight of larvae. The study of the interaction between EPN and RPW could be crucial understanding the mode of action of EPN in the different instars and the reason for the response to different doses.

Poster Papers Wednesday, 16:30-18:30

Viruses

Poster / Viruses, Wednesday 16:00 **V1 STU**

Dissecting the response to White Spot Syndrome Virus in non-model host decapod taxa in non-model environmental scenarios

Lauren S. Hall[1], Dr. Chris Hauton[2], Dr. Grant Stentiford[3]
[1,2]National Oceanography Centre Southampton, University of Southampton, European Way, Southampton, SO14 3ZH, UK, [3]CEFAS, The Nothe, Barrack Road, Weymouth, Dorset, DT4 8UB, UK
Address for correspondence: lsh203@soton.ac.uk

White spot syndrome virus (WSSV) causes high mortality in decapod crustaceans, notably penaeid shrimps. In response to the increasing geographical range of reported cases since the 1990s, all decapod crustaceans are listed in European legislation as potentially susceptible. Recent work by our group supports the wide range of potential host species when exposure to the virus occurs at European ambient conditions. However, the *relative* susceptibility to disease is highly variable between host taxa. Specifically, a particularly low-level susceptibility to disease is reported for the European shore crab, *Carcinus maenas* (L.). We present a comparative study of the molecular pathogenesis of WSSV infection between juvenile *Carcinus maenas* and the highly-susceptible European lobster *Homarus gammarus* (L.). A selection of viral (*ie1, dnapol, vp28*), immune (*carcinin, amp, peroxinectin, prophenoloxidase*) and endogenous reference genes (*β-actin, ubiquitin, tubulin, gapdh, eef1a* and *ppia*) are selected to quantify the immune host response and viral gene expression using sensitive qPCR techniques. This work will establish whether resistance in *Carcinus maenas* is a function of species differences or ontogenetic stage and the point in the viral replication cycle at which host resistance is exhibited. Furthermore, this study will identify possible molecular mechanisms by which resistance to WSSV is established.

Poster / Viruses, Wednesday 16:00 **V2**

Possible origin of a nucleopolyhedrovirus in winter moth populations in Massachusetts

Woojin Kim[1]; Joseph S. Elkinton[1]; George H. Boettner[1] ; John P. Burand[1,2]
Departments of [1]Plant, Soil and Insect Sciences, [2]Microbiology, University of Massachusetts – Amherst, Amherst, MA 01003 USA.
Address for correspondence: jburand@microbio.umass.edu

The winter moth, *Operophtera brumata*, originally from Europe, has recently invaded eastern Massachusetts. This insect has caused widespread defoliation of many deciduous tree species and severely damaged a variety of crop plants in the infested area including apple, strawberry and especially blueberry. Recently, diagnostic PCR with polyhedrin specific primers was used to detect O. brumata nucleopolyhedrovirus (OpbuNPV) in larvae and pupae collected in Massachusetts, USA. Here we used newly designed p74 specific primers to amplify an 1116 bp partial sequence of the p74 gene of OpbuNPV isolates from British Columbia, Canada, Norway the United Kingdom, and Massachusetts, USA. A phylogenetic analysis of these sequence data indicated that the OpbuNPV isolated the USA is more closely related to the isolates from Norway than virus recovered in the UK of British Columbia suggesting that this virus may have originated from insect populations which moved into North America from Northern Europe.

Poster / Viruses, Wednesday 16:00 **V3 STU**

Adaptation of an AcMNPV population to *Trichoplusia ni* and *Spodoptera exigua*

Aurélien Chateigner[1]; Davy Jiolle[1]; Carole Labrousse[1]; Annie Bézier[1]; Elisabeth Herniou[1]
[1]Institut de Recherche sur la Biologie de l'Insecte, UMR CNRS 6035, Université François Rabelais de Tours, Faculté des Sciences et Techniques, Avenue Monge - Parc Grandmont 37200 Tours France.
Address for correspondence: aurelien.chateigner@gmail.com

Baculoviruses adapt to environmental changes by natural selection mechanisms. For a virus, the infected cell is its defined and obligatory environment, its ecological niche. Since different host species represent different niches, we can expect a given virus population to have different virulence towards different host species. This should be reflected by host susceptibility. Our AcMNPV population bioassays in 6 species showed that different magnitude order, ranging from 200 to 1000 billions, of OBs were necessary to obtain the LD90 *Trichoplusia ni, Spodoptera exigua, Manduca sexta, Chrysodeixis chalcites, Agrotis ipsilon* and *Mamestra brassicae*. To study the AcMNPV environmental adaptation, we compare its virulence evolution in two different host species, *T. ni* and *S. exigua*. The experimental evolution set up consists in 5 infections passages, realised in 10 replicates. We started the first passage by feeding 3rd instar caterpillars 2500 OBs of a polymorphic AcMNPV strain (above LD50). OBs were harvested and resuspended in equal volumes, so that proportional amount could be fed to the larvae in the subsequent passages. From these infections, we observe the lethal time (LT50) as an indicator of virulence. The results showed the adaptation trajectories taken by the virus population to adapt to specific host species.

Poster / Viruses, Wednesday 16:00 **V4**

Genetic variation and biological activity of nucleopolyhedrovirus samples from larvae *Heliothis virescens, Helicoverpa zea,* and *Helicoverpa armigera*

Daniel L. Rowley[1]; Holly J. R. Popham[2]; Robert L. Harrison[1]
[1]Invasive Insect Biocontrol and Behavior Laboratory, Plant Sciences Institute, USDA Agricultural Research Service, 10300 Baltimore Avenue, Beltsville, Maryland 20705, USA, [2]Biological Control of Insects Research Laboratory, USDA Agricultural Research Service, 1503 S. Providence Road, Columbia, Missouri 65203, USA
Address for correspondence: Robert.L.Harrison@ars.usda.gov

To assess the diversity and relationships of baculoviruses found in insects of the heliothine pest complex, a PCR-based method was used to classify 90 samples of nucleopolyhedrovirus (NPV; Baculoviridae: *Alphabaculovirus*) obtained worldwide from larvae of *Heliothis virescens* (Fabricius), *Helicoverpa zea* (Boddie), and *Helicoverpa armigera* (Hübner). Partial nucleotide sequencing and phylogenetic analysis of three highly conserved genes (*lef-8, lef-9,* and *polh*) indicated that 67 of these samples contained isolates of the *H. zea-H. armigera* single nucleopolyhedrovirus (Hz/HaSNPV) species group. Eighteen of the samples contained isolates of a multiple NPV from *H. armigera*, HearMNPV, and five of the samples contained isolates of *Autographa californica* MNPV (AcMNPV). The Hz/HearSNPV isolates occurred in two separate groups of HearSNPV variants and a third group of HzSNPV variants; this organization was confirmed by sequencing and analysis of an additional seven loci (*orf5/orf5b, hr3-orf62, orf26, orf79, orf124/orf117a, orf42,* and a part of the region between *hr2* and *hr3*). Some of the samples contained isolates of

more than one virus. In bioassays of a selection of isolates against *H. zea*, the commercially available Gemstar isolate of HzSNPV killed larvae faster than most other Hz/HaSNPV and HearMNPV isolates. Gemstar and two HearMNPV isolates exhibited significantly higher LC_{50}s than the Hz/HearSNPV isolates tested. This study expands significantly on what we know about the variation of heliothine NPV populations and provides novel information on the distinct groups in which these NPVs occur.

Poster / Viruses, Wednesday 16:00 **V5STU**

Influence of polyhedra morphology on the virulence of *Autographa californica* nucleopolyhedrovirus

Jae Bang Choi; Won Il Heo; Tae Young Shin; Sung Min Bae; Soo Dong Woo
Department of Agricultural Biology, Chungbuk National University, Cheongju, 363-763, Korea
Address for correspondence: sdwoo@cbnu.ac.kr

The polyhedrin is responsible to form polyhedra of nucleopolyhedrovirus (NPV) and highly conserved in most completely sequenced in lepidopteran NPVs. Previously, we have reported that the substitution of polyhedrin of *Autographa californica* NPV(AcNPV) with that of *Spodoptera exigua* NPV(SeNPV) or *Bombyx mori* NPV(BmNPV) result the change of polyhedra morphology. In this study, we investigated the influence of changed polyhedra morphology to the virulence of AcNPV. The recombinant AcNPVs were propagated in *Spodoptera frugiperda* clone 9, 21 cells and *S. exigua* larvae. Each collected recombinant polyhedra were used in bioassays using *S. exigua* larvae. The recombinant AcNPVs show that difference virulence according to the polyhedra morphologies. Internal and external morphological features of each recombinant AcNPV were also compared on the electron microscope. Our results suggest that the morphology of polyhedra influence the virulence of NPV and is well worth considering for the development viral insecticide.

Poster / Viruses, Wednesday 16:00 **V6**

Characterization of six Many Polyhedra variants of *Anticarsia gemmatalis* MNPV

Souza, M. Lobo, Sihler, W.;Brito, A. Fernandes
[1]Embrapa Recursos Genéticos e Biotecnologia - Parque Estação Biológica - PqEB - Av. W5 Norte (final) Caixa Postal 02372 - Brasília, DF - Brasil - 70770-917
Address for correspondence: marlinda@cenargen.embrapa.br

Baculovirus are insect viruses used in pest control programs as an alternative to chemical pesticides. Baculovirus *in vivo* production is already a well established procedure. However, there is a great limitation for the *in vitro* production due to accumulation of Few Polyhedra (FP) mutants after virus serial passage in cell culture. This event results in the decrease of the occluded viral particles and leads to loss of virulence. Recently, stable virus selection, called Many Polyhedra (MP) has been presented as a possible strategy for large scale in *vitro* production. In previous studies, six Many Polyhedra (MP) variants were selected after seven successive passages of the *Anticarsia gemmatalis* nucleopolyhedrovirus (*Ag*MNPV-2D) in cell culture. In the present work, these variants were analyzed according to their DNA restriction profile. Initially, BTI-Tn-5B1-4 cells were infected with the virus and the supernatant collected 5 days after infection. The budded viruses (BVs) produced were purified in a 25% sucrose cushion and DNA was phenol purified. As expected, DNA restriction enzymes analysis showed no difference when MPs variants were compared to the *Ag*MNPV-2D. Similarly, the infected cells protein profile of these variants showed no changes. The many polyhedra formation after successive cell passages, together with the protein and DNA profiles data, indicate that the six MPs kept the wild virus pattern. Analysis by electron microscopy and determination of budded virus titer are currently in progress to proceed the characterization of these viruses

Poster / Viruses, Wednesday 16:00 **V7 STU**

Novel interactions of a cypovirus in the *Heliothis virescens* and *Campoletis sonorensis* host-parasitoid system.

Juliane Deacutis; Philip Houtz; Bruce Webb
Department of Entomology, S225 Ag Science North, University of Kentucky, Lexington, KY 40546-0091
Address for correspondence: bawebb@uky.edu

Some viruses, notably the ascoviruses, are reliant upon parasitoid wasps for horizontal transmission between their lepidopteran hosts. Other viruses, the polydnaviruses, are obligate symbionts of parasitoid wasps, existing in highly commensal and evolved relationships with their parasitoid hosts. Among the viruses associated with parasitoid wasps, polydnaviruses are considered beneficial and are essential aids to wasps in the parasitism of their lepidopteran host. In contrast, the impact of ascoviruses within the wasp-host system can be detrimental to both the wasp and its lepidopteran host. There are many viruses, however, that are associated with parasitoid wasps or the lepidopteran host, and little is known about their impact on the host-parasitoid system. We have recently discovered two variants of a type 5 cypovirus in both a parasitoid wasp, *Campoletis sonorensis* and in the wasp's conventional host, *Heliothis virescens* larvae. This small RNA virus has little overt effect on its lepidopteran host while the impact of cypovirus infection on wasp parasites has not been examined. We conducted studies to examine the distribution, localization and pathologies of these viral variants in *C.* sonorensis and *H.* virescens. We describe here a novel virus which appears to interact with the host-parasitoid system in a fundamentally new and intriguing way.

Poster / Viruses, Wednesday 16:00 **V8 STU**

The characterization of a novel cypovirus in a parasitoid-host relationship

Philip, L. Houtz[1]; Juliane, M. Deacutis[2]; Bruce, A. Webb[3]
[1]140 Hunter's Rest Lane, Lexington, KY 40515; [2]3546 Creekwood Dr. #20, Lexington, KY 40502 US; [3]S225 Agriculture Science Center North, Lexington, KY 40546-0091
Address for correspondence: Plhout2@uky.edu

Polydnaviruses (PDVs) in parasitoid wasps are an iconic example of the curious and expansive realm of viruses that directly benefit their host species. In this case, PDVs allow such wasps to overcome the defense responses of their host organisms. In the parasitoid/host relationship between *Campoletis sonorensis* and *Heliothis virescens* we have recently discovered two variants of another virus, present in both the parasitoid wasp and its caterpillar host. We have identified this new virus as a cypovirus (CPV), and suggest that, while it appears to have little effect upon the caterpillar hosts, it may be responsible for a high mortality rate that we have observed in our parasitoid wasps, which become exposed to the virus while feeding on their hosts. The activity of the cypovirus in the PDV lifecycle provides us with a unique chance to study the complex relationship that exists between these viruses and their development as biological weapons in their primary hosts.

Poster / Viruses, Wednesday 16:00 **V9 STU**

Generation of an orally infective recombinant AgMNPV with improved bioinsecticidal activity

Santiago Haase[1]; M. Leticia Ferrelli[1]; Ricardo Salvador[2]; Marcelo F. Berretta[2]; Alicia Sciocco-Cap[2] &Víctor Romanowski[1]
[1]IBBM-UNLP-CONICET, Universidad Nacional de La Plata, La Plata; [2]IMyZA-INTA, Castelar; Argentina.

Address for correspondence: *santiagohaase@hotmail.com*

AgMNPV (*Anticarsia gemmatialis* Nucleopolyhedrovirus), the most widely used viral bioinsecticide, has been successfully applied for control of its host, the velvetbean caterpillar (*Anticarsia gemmatalis*). However, the use of this baculovirus presents among its limitations a slow speed of kill in temperate climates. In this context, our group developed a system for the genetic modification of AgMNPV, based on double homologous recombination between the linearized viral genome and a transfer vector. Recombinant viruses obtained using transfer vectors previously constructed provided adequate expression levels of heterologous gene. However, propagation of the recombinants in cell culture resulted in viruses not infective *per os*, probably due to genetic rearrangements. Therefore a new generation of transfer vectors was constructed. The system was first checked using a new vector bearing the green fluorescent protein gene (*egfp*). A rAgMNPV-*egfp* was generated and its oral infectivity and eGFP expression were confirmed using 3rd instar *A. gemmatalis* larvae. Using this system an insect-selective neurotoxin (*tox34*) gene from *Pyemotes tritici* was used to construct rAgMNPV-*tox34*. Expression of the toxin by the virus causes a reduction in the time required to kill the host insect. Both rAgMNPV were characterized by PCR, restriction pattern analysis and SDS-PAGE of infected cells. In conclusion, rAgMNPV-*egfp* can be used as a tool for basic research and rAgMNPV-*tox34* exhibits an increased biopesticidal activity.

Poster / Viruses, Wednesday 16:00 **V10**

Comparative analysis of mononucleotide repeat (MNR) sequences in the genomes of baculoviruses

Xiao-Wen Cheng[1], Samuel T. Murakami[1], Emily E. Schmidt[2], David C. Ream[1] and Chun Liang[2] and Iddo Friedberg[1]
[1]Department of Microbiology, Miami University, Oxford, Ohio 45056 USA; [2]Department of Botany, Miami University, Oxford, Ohio 45056 USA
Address for correspondence: Chengx@muohio.edu

Baculoviruses are insect specific double-stranded DNA viruses with a genome size in the range of 80-180 kb. The baculovirus genome is replicated by its viral DNA polymerase in the nuclei of permissive insect cells. Replication of the baculovirus genome by the viral DNA polymerase often generates errors on mononucleotide repeat (MNR) sequences due to replication slippage resulting in inactivation of genes that affects different stages of virus infection cycles. Forty eight baculovirus genomes have been sequenced and published to date. We developed a computer program Repeater and a website-based program for the search of MNRs in the baculovirus genomes. The MNR occurrences of the 48 baculovirus genomes were retrieved by the computer programs, analyzed and compared. It was found that the MNR occurrences of baculovirus genomes are not congruent with the baculovirus genome sizes. Even though, the average A/T content of baculovirus is 58.9% in the range of 42.5-67.6%, the A/T MNR occurrence is significantly higher than the G/C MNR occurrence and the A7/T7 is the most frequent MNRs in all the baculovirus genomes examined. More MNRs in coding regions than in intergenic regions are found in the 45 baculovirus genomes, whereas more MNRs in intergenic regions than coding regions occur in the other 3 baculovirus genomes. The MNR occurrences in different classes of baculovirus genes, such as immediate early genes, late and very late genes, also showed differences among difference baculovirus genomes suggesting the distribution and frequency of MNR in different types are unique to each baculovirus.

Poster / Viruses, Wednesday 16:00 **V11 STU**

Complete comparative genomic analysis of two strains of *Bombyx mori* nucleopolyhedrovirus isolated in Korea

Hee Jung Kim[1]; Won Il Heo[1]; Jae Bang Choi[1]; Sung Min Bae[1]; Bit na rae Yun[1]; Jun beom Lee[1]; Soo-Dong Woo[1]
[1]Department of Agricultural Biology, Chungbuk National University, Cheongju, 363-763, Korea
Address for correspondence: sdwoo@cbnu.ac.kr

The four genetically distinct isolates have been identified previously from *Bombyx mori* nucleopolyhedroviruses (BmNPVs) isolated in Korea. To further understand the complex of viruses infecting *Bombyx mori*, the genome of BmNPV-K1 and K4 strains was completely sequenced and analyzed in comparison with the genome of other sequenced baculoviruses including previously reported BmNPV. BmNPV-K1 consisted of 127,542 bp and 133 open reading frames (ORFs) of 150 nucleotides or longer with minimal overlap have been identified. In contrast, BmNPV-K4 consisted of 128,615 bp and 134 ORFs. Although gene arrangement is virtually identical, the genome of BmNPV-K4 is 1,073 bp longer than BmNPV-K1. This was related to the more existence of *bro* genes in BmNPV-K4. To investigate the relationship between BmNPV-K1 and K4, phylogenetic analysis with each member of the paired ORFs was performed. The sequence data suggest that BmNPV-K1 and BmNPV-K4 are closely related but have diverged and evolved into two separate strains. This was study to identify highly related but separately evolving viruses in the same insect host and geographic location. We are currently comparing the differences of these BmNPV genomes to elucidate characteristics of each virus.

Poster / Viruses, Wednesday 16:00 **V12**

Identification of soybean aphid viruses using Next Generation sequencing technology

Sijun Liu, Diveena Vijayendran, Bryony C. Bonning
Department of Entomology, Iowa State University, Ames, IA 50011 USA
Author for correspondence: bbonning@iastate.edu

The soybean aphid, *Aphis glycines* Matsumura, is a major pest of soybean in North America. Management of the soybean aphid has cost an estimated $1.6 billion over the last 10 years. Current management relies primarily on the application of classical chemical insecticides, which deleteriously impact arthropod natural enemies. Our goal is to identify and characterize viruses of the soybean aphid which have potential for use in soybean aphid management. The soybean aphid transcriptome was sequenced using Illumina/Solexa short read sequencing and the data screened for viral sequences. Three viruses were identified from the transcriptome. We are using bioinformatics tools and protein structure prediction programs to characterize the three soybean aphid viruses, and RT-PCR to obtain the complete genome sequences. One of the viruses, *Aphis glycines* virus (AGV) is estimated to have a 5 kb single stranded RNA genome and to form a 30 nm particle. The RNA-dependent RNA polymerase of this virus is closely related to that of *Euprosterna elaeasa* virus (EeV: Tetraviridae) and *Drosophila* A virus (DAV: unclassified). AGV appears to have a 100% vertical transmission rate and has also been detected by RT-PCR in laboratory colonies of two other aphid species, the bird cherry-oat aphid, *Rhopalosiphum padi* (Linnaeus) and the green peach aphid, *Myzus persicae* (Sulzer).

Poster / Viruses, Wednesday 16:00 **V13**

Genome structure of a nucleopolyhedrovirus infecting *Abagrotis reedi*, a cutworm pest of vineyards.

Martin A. Erlandson[1]; Ajaykumar Maghodia[1]; Joan Cossentine[2]; Tom Lowery[2]; David A. Theilmann[2]
[1]Agriculture and Agri-Food Canada, Saskatoon Research Centre, 107 Science Place, Saskatoon, SK S7N 0X
[2]Agriculture and Agri-Food Canada, Pacific Agriculture Research Centre, 4200 Highway 97, Summerland, BC V0H 1Z0 Canada.
Address for correspondence: erlandsonm@agr.gc.ca

A species complex of climbing cutworms, mainly in the genera *Euxoa* and *Abagrotis*, are pests of grape vineyards in the Okanogan region of Canada. A series of *Alphabaculovirus* strains were isolated from single-infected-larva samples from cutworm populations in vineyards across the Okanogan region. One isolate was highly infectious for *Abagrotis reedi* larvae. Occlusion bodies from this isolate were purified and genomic DNA extracted for 454 DNA sequence analysis. The resulting DNA sequence assembled into three large contigs. PCR primers were designed to span the two intervening gaps and the PCR amplicons were sequenced using Sanger sequencing procedures. The resulting genomic sequence was 157,108 nt which is predicted to encode 151 ORFs and contained eight homologous repeat regions. Blast analysis indicated that the *Abragrotis reedi* nucleopolyhedrovirus (AbreNPV) ORFs typically had the highest homology to *Agrotis ipsilon* NPV (AgipNPV) ORFs. In addition the AbreNPV genome was largely collinear with that of AgipNPV. Phylogenetic analysis showed that AbreNPV is part of the previously identified clade of group II NPVs that includes viruses from *Agrotis* and *Spodoptera* host species. The deep 454 DNA sequencing approach produced greater than 40 fold coverage and identified two major genotypes within the AbreNPV population isolated from the single-infected-larva isolate.

Poster / Viruses, Wednesday 16:00 **V14**

Proteomics analysis of the occlusion derived virus (ODV) of *Neodiprion abietis* nucleopolyhedrovirus (NeabNPV)

Shannon Escasa[1], Misha Demidovich[1], Christopher Lucarotti[2], Peter Krell[3] and Basil Arif[1]
[1]Laboratory for Molecular Virology, Great Lakes Forestry Centre, CFS. [2]Atlantic Forestry Centre, CFS. [3]Dept. of Cellular and Molecular Biology, University of Guelph.
Address for correspondence: barif@nrncan.gc.ca

The balsam fir sawfly, *Neodiprion abietis* (Harr.) is a common defoliator of balsam fir throughout North America. The potential for controlling this pest species is important and can be successful with a greater understanding of the biology of the virus, *Neodiprion abietis* nucleopolyhedrovirus (NeabNPV), naturally pathogenic to its host. The occlusion-derived virus (ODV) is the viral form responsible for primary infection in its host. Identifying the proteins involved and associated with the ODV is a key component to understanding the biology of NeabNPV. Techniques and methods used to identify the proteins include SDS-PAGE, in-gel trypsin digestion, LC-MS/MS, QTPOF and specialized software such as Scaffold and Mascot. As a result, a total of 29 proteins were found to be associated with the ODV, most having homologues to other baculovirus ODV proteins. Twenty of these proteins include: VP91, PIF-1, P49, ODV-C42, ODV-EC43, PIF-5 (ODV-E56), VP1054, VP39, 38K, P33, ODV-E27, POLH, GP41, PIF-3, PIF-2, NEAB40(Ac68), PIF-4 (ODV-E28), P74, P6.9 and ODV-E18. In addition, nine novel proteins were discovered encoded by NEAB13 (81 kDa), NEAB20 (20.0 kDa), NEAB33 (12.0 kDa), NEAB54 (22.0 kDa), NEAB70 (7.0 kDa), NEAB72 (8.0 kDa), NEAB82 (18.0 kDa), NEAB89 (8.0 kDa), and NEAB90 (36 kDa). Eight of the novel proteins only had homologues to the two other sequenced hymenopteran baculoviruses, *Neodiprion lecontei* (NeleNPV) and *Neodiprion sertifer* (NeseNPV), while NEAB70 was unique to NeabNPV and did not have homologues to any other baculoviruses. These are possibly new proteins and their function is yet to be determined.

Poster / Viruses, Wednesday 16:00 **V15**

A naturally occurring mutant of *Spodoptera frugiperda* multiple nucleopolyhedrovirus reveals the existence of a new alphabaculovirus *per os* infectivity factor (PIF-6)

Oihane Simón[1]; Leopoldo Palma[1,2], Trevor Williams[3]; Miguel López-Ferber[4]; Primitivo Caballero[1,2]
[1]Instituto de Agrobiotecnología, CSIC, Gobierno de Navarra, 31192 Mutilva Baja, Navarra, Spain, [2]Departamento de Producción Agraria, Universidad Pública de Navarra, 31006 Pamplona, Spain, [3]Instituto de Ecología AC, Apartado Postal 63, Xalapa, Veracruz 91070, México, [4]Laboratoire de Gènie de l'Environnement Industriel, Ecole des Mines d'Alès, 6 avenue de Clavières, 30319, Alès Cedex, France.
Address for correspondence: oihane.simon@unavarra.es

The Nicaraguan population of *Spodoptera frugiperda* multiple nucleopolyhedrovirus, SfMNPV-NIC, is structured as a mixture of genotypes. SfMNPV-C, -D and –G pure genotypes are incapable of oral transmission. In SfMNPV-C and –D the non-transmissible phenotype is due to the absence of *pif1* and *pif2* genes. The complete sequence of the SfMNPV-G genome was determined identify possible factors involved in this phenotype. Two deletions of 4,860 bp (22,364-27,225) and 60 bp (119,759-119,818) were observed in SfMNPV-G genome compared with that of SfMNPV-B, the predominant complete genotype (132,954 bp). However no genes homologous to previously describe *per os* infectivity factors were detected in these deletions. Significant differences were found in the nucleotide sequence in *sf58* gene (unknown function) that produced changes into the amino acid sequence and the predicted secondary structure of the protein. This gene is conserved only in alphabaculoviruses. To determine the role of *sf58* in the peroral infectivity a deletion mutant was constructed using bacmid technology. The OBs of the deletion mutant (Sf58null) were not orally infectious for *S. frugiperda* larvae, although Sf58null DNA and ODVs were as infective as SfMNPV bacmid DNA and ODVs in intrahemocelically infected larvae or cell culture, indicating that defects in ODV or occlusion body morphogenesis were not involved in the observed loss of infectivity. The addition of Tinopal or the presence of the orally infectious SfMNPV-B genotype in mixtures with SfMNPV-G did not recover Sf58null OB infectivity. According to these results *sf58* is a new *per os* infectivity factor (PIF-6) present only in alphabaculoviruses.

Poster / Viruses, Wednesday 16:00 **V16 STU**

Analysis of a novel transactivator BmNPV p15

Yukimi Akatsuka; Takumi Abe; Yasuyuki Nishijima; Chikako Ono; Ken Sahara; Shin-ichiro Asano; Hisanori Bando
Division of Applied Bioscience, Graduate School of Agriculture, Hokkaido University, Sapporo 060-8589, Hokkaido, Japan
Address for correspondence: yukimi-a@abs.agr.hokudai.ac.jp

Recently, we reported that a 35S promoter of cauliflower mosaic virus (35Sp) was activated by the viral RNA polymerase in the cells infected with *Autographa californica* NPV (AcMNPV) (Abe *et al*., 2005). Subsequently, we found that 35Sp could be activated by several genes of *Bombyx mori* NPV (BmNPV) in a transient assay (unpublished data). In the experiment, *p15* was identified as a BmNPV gene having the ability to activate 35Sp without expression of viral RNA polymerase. The *p15* product (p15) has been known as a putative capsid protein (Lu and Iatrou, 1996) rich in positive charged amino acids but functionally obscure so far. Our observations suggested that *p15* coded for a novel transactivator for viral genes. We then examined the effects of *p15*

on the activities of early and late promoters of BmNPV in the transient assay using the reporter plasmids containing each promoter. All of the promoters used in this experiment were activated by cotransfection with a *p15*-expressing plasmid. Further experiments using the deletion mutants of *p15* suggested that p15 had multiple domains having additive effects in the viral promoter activation. The study on the functions of p15 in the viral replication using a *p15*-knockout virus is in progress.

Poster / Viruses, Wednesday 16:00 **V17**

Characterization of new active transposons isolated from insects

Mariano N. Belaich, Matías J. Garavaglia, Solange A.B. Miele, Cecilia S. Turco, Diego L. Mengual Gomez, Vanina A. Rodriguez and Pablo D. Ghiringhelli.
LIGBCM-AVI, Departamento de Ciencia y Tecnología, Universidad Nacional de Quilmes. Roque Saenz Peña 352, Bernal, Pcia. Buenos Aires, Argentina
Corresponding author: pdg@unq.edu.ar

Transposons are mobile DNAs spread in most organisms including some viruses. The ability of these sequences in mobilizing from one physical position to another can be a decisive factor in evolution processes due to genomic rearrangements (gene interruption, deletions, inversions, translocations, etc.). In Eukarya, transposable elements (TEs) are a significant percentage of genomes showing a great diversity in gene content, size and mechanism of transposition. According to the above, TEs are classified into two main groups: Class I (retrotransposons) and Class II (DNA transposons). Insect cells are the best system to study and produce baculoviruses, a pathogen used as bioinsecticide, protein expression system and gene therapy or vaccine vectors. These viruses have big dsDNA genomes and structural mutations produced by transposition processes could be the main force of their evolution. With the aim to discover and characterize new active transposons from insects, we transfected prokaryotic plasmids in insect cell lines (Sf9 and Sf21 from *Spodoptera frugiperda*, Hi5 from *Trichoplusia ni* and UFL-Ag-286 from *Anticarsia gemmatalis*) and then we recovered modified plasmids with DNA insertions by *Escherichia coli* transformation. The proposed strategy has allowed isolating and sequencing 4 TE´s never before described and Piggybac, a transposon with many applications in biology.

Poster / Viruses, Wednesday 16:00 **V18 STU**

Construction of novel baculovirus expression vector system by fusion of partial polyhedrin

Sung Min Bae; Bit Na Rae Yun; Jun Beom Lee; Hee Jung Kim; Soo Dong Woo
Department of Agricultural Biology, Chungbuk National University, Cheongju, 363-763, Korea
Address for correspondence: sdwoo@cbnu.ac.kr

Polyhedrin is the major component of the nuclear viral occlusions produced during replication of the baculovirus *Autographa californica* multicapsid nuclear polyhedrosis virus (AcMNPV). To enhance the expression level of baculovirus vector system, we constructed several fusion vectors using various fragments of the polyhedrin. The polyhedrin fragments were genetically fused to the enhanced green fluorescent protein (eGFP) under the control of polyhedrin promoter, and their expressions were analyzed in Sf21 insect cells. Expression of the fusion protein was identified by SDS-PAGE and Western blot analysis using anti-GFP and anti-Polyhedrin. The expression level of eGFP was markedly increased by the fusion of partial polyhedrin. Also, the fluorescence intensity of fusion proteins was higher than that of non-fusion protein. Confocal laser scanning microscopy demonstrated that fusion proteins were localized to the cytosol or nucleus of insect cells. In additional, the glycoprotein E2 (gE2) of classical swine fever virus (CSFV) expressed by these vectors was dramatically increased and its immunogenicity was proofed using experimental animal guinea pigs that were immunized with the partial polyhedrin containing gE2. This study provides a new option for the higher expression of useful foreign recombinant protein by using the partial polyhedrin in BEVS.

Poster / Viruses, Wednesday 16:00 **V19**

Construction of the full-length cDNA clone of *Ectropis oblique* picorna-like virus

Yuanyang Hu; Meijuan Lin; Congyi Zheng; Jiamin Zhang
State Key Laboratory of Virology, College of Life Sciences, Wuhan University, Wuhan, Hubei 430072 China
Address for correspondence: yyhu@whu.edu.cn

Ectropis oblique picorna-like virus (EoPV) is an insect RNA virus that causes a lethal granulose infection in the larvae of tea looper (*Ectropis oblique*). EoPV is the first identified insect picorna-like virus in China. The genome of EoPV is single strand RNA, which is polyadenylated and contains a single large open reading frame (nt 391-9351) that encodes a 2987-aa polyprotein. In this polyprotein, structural and nonstructural proteins are located at its N- and C-terminal regions, respectively. Its 5'-UTR is 390 nt in length and its 3'-UTR is 43 nt in length. EoPV has been classified as a member of the genus *Iflavirus* in family *Iflaviridae*. To study the replication mechanism of EoPV RNA, we extracted the viral total RNA from dead larvae of the tea loopers infected by EoPV. Five pairs of primers were designed according to the published EoPV sequence and were utilized to amplify five overlapping fragments, which cover EoPV genome. These fragments were subcloned to pET-28a vector and verified by sequencing and restrictive digestion. Compared with the published sequence, this clone contains an 8-amino acid mutation and a 1-amino acid deletion. This study represents the first step toward understanding the mechanism of EoPV RNA replication.

Poster / Viruses, Wednesday 16:00 **V20**

Study of late expression factors of *Spodoptera frugiperda* nucleopolyhedrovirus in the permissive insect cell line Sf-9

Marcelo F. Berretta[1]; M. Gabriela López[2]; Alicia Sciocco-Cap[1] & Víctor Romanowski[3]
[1]IMYZA-CICVyA, Instituto Nacional de Tecnología Agropecuaria (INTA), CC 25 (1712) Castelar, Buenos Aires, Argentina, [2]IB-CICVyA-INTA, [3]IBBM-CONICET-UNLP, Facultad de Ciencias Exactas, Universidad Nacional de La Plata, calle 49 y 115, (1900) La Plata, Buenos Aires, Argentina.
Address for correspondence: mberretta@cnia.inta.gov.ar

Baculovirus expression of late genes depends on the activity of a group of genes, collectively known as late expression factors (*lefs*), that includes components of the DNA replication machinery and the viral RNA polymerase complex. *Lefs* have been more extensively studied in the model virus AcMNPV, a group I alphabaculovirus. Nineteen AcMNPV *lefs* were required to achieve optimal expression from a late promoter in a transient expression assay in Sf-21 cells, although other genes may also influence late expression in the context of the viral infection. Since homologs were not identified for all AcMNPV *lefs* in other baculovirus lineages it is possible that functional homologs occur in other species. We are interested in studying *lefs* of SfMNPV, a group II alphabaculovirus. SfMNPV infects productively Sf-21 and Sf-9 cell lines, as AcMNPV does. However, SfMNPV lacks homologs of AcMNPV *lefs ie-2, lef-12* and *p35*. We constructed a SfMNPV *lef* library and tested the ability of this set of 16 genes to activate a late promoter in a reporter plasmid cotransfected in Sf-9 cells. These SfMNPV *lefs* were not functional in this assay; therefore, in order to identify putative genes able to complement

the SfMNPV *lef* library, we initiated a genome-wide screening. In a preliminary survey DNA fragments that account for approximately one third of the genome and do not encompass any previously known *lefs* supported a significant increase of reporter activity. This result warrants further dissection of these regions to ultimately identify genes putatively involved in late expression in this virus-host system.

Poster / Viruses, Wednesday 16:00 **V21**

Stability analysis of Sf-caspase-1 in Sf9 cells

Zhongfu Ying, Ao Li, Meijin Yuan, Kai Yang, Yi Pang
State Key Laboratory of Biocontrol, Sun Yat-sen University, Guangzhou 510275, China
Address for correspondence: yangkai@mail.sysu.edu.cn

Sf-caspase-1 is the principal effector caspase in *Spodoptera. frugiperda* cells. Like the caspases in other organisms, Sf-caspase-1 is processed by upstream caspases to form an active heterotetramer that is composed of the p19 and p12 subunits. In mammal cells, it has been demonstrated that the active form of effector caspases was highly labile and was rapidly turned over relative to its proenzyme form. Considering that the regulation of active caspases is crucial for cellular viability, we investigated whether the subunits of Sf-caspase-1 were regulated by a degradative mechanism, as has been observed in several other organisms. Sf9 cells were transiently transfected with plasmids encoding different Sf-caspase-1 polypeptides: the pro-Sf-caspase-1 peptide (p37), a peptide that lacks the prodomain (p31), a peptide that contains the large subunit and the prodomain (p25), the large subunit peptide (p19), and the small subunit peptide (p12). Microscopy and Western blot analyses revealed that p12, p19 and p25 were unstable in the transfected cells, in contrast to p37 and p31. Lactacystin treatment increased the accumulation of the p19 and p12 subunits, which suggests that the degradation is performed by the ubiquitin-proteasome system. The active and the intermediate form of Sf-caspase-1 were also unstable and degraded by ubiquitin-proteasome system.

Poster / Viruses, Wednesday 16:00 **V22**

The impact of silencing AcMNPV ORF33 on sxpressing integral membrane proteins by baculoviruses

Tamer Z. Salem[1,3], and Suzanne M. Thiem[1,2]
[1]Department of Entomology, [2]Department of Microbiology and Molecular Genetics, Michigan State University, East Lansing, MI 48824, U.S.A., [3]Department of Microbial Molecular Biology, AGERI, Agricultural Research Center, Giza 12619, Egypt
Address for correspondence: smthiem@msu.edu

Baculovirus expression of integral membrane proteins (IMPs) shows promise but could be improved. Because virus infection alters host cell function, we reasoned that functions of specific virus genes could interfere with IMP synthesis and/or processing. To identify candidate genes, an RNA interference (RNAi) screen was performed in recombinant *Autographa californica* nucleopolyhedrovirus (AcMNPV)-infected Sf21 cells. Recombinant viruses expressed model IMPs from the polyedrin promoter as carboxy-terminal enhanced green fluorescent protein (eGFP) fusions. A total of 83 AcMNPV genes that have not been confirmed as essential were targeted in this screen. Several candidates were identified based on increased expression of the eGFP reporter when their expression was knocked down. The highest enhancement level among them (~ 50 %) was achieved by knocking down ORF33 expression. When recombinant viruses with a full-length deletion of ORF33 (vAcΔ33), an ORF33 insertional mutation, the upstream ORF32 (vFGF) with an insertional mutation, or an ORF32/33 double mutation were tested, expression levels were not enhanced. There are several possible explanations for the differences between the RNAi and gene deletion results. One is that the ORF33 gene product needs to be maintained in the cell at a lower level, as RNAi does not reduce expression levels 100%. Another is that expression of an adjacent ORF with an overlapping gene transcript was reduced as ORF33 was targeted and is responsible for enhanced expression. It is also possible that the dsRNA targeting ORF33 has an off-target effect on host cell transcripts.

Poster / Viruses, Wednesday 16:00 **V23 STU**

Baculovirus as a gene delivery vector for ischaemia reperfusion injury

Elisabetta Locanto[1]; Richard B. Hitchman[2]; Linda A. King[1]
[1]Oxford Brookes University, Insect Virology Research Group, School of Life Sciences, Oxford, UK.
[2]Oxford Expression Technologies Ltd, Oxford, UK;
Address for correspondence: elisabetta.locanto-2010@brookes.ac.uk

Ischaemia reperfusion injury (IRI) commonly occurs during organ transplantation, is associated with hypoxia and free radicals formation and is associated with organ failure following transplantation. Evidence suggests that the mitochondrial manganese superoxide dismutase (Mnsod) gene is involved in cellular protection against free radical damage. Mammalian viruses carrying the Mnsod gene have been used in gene therapy to ameliorate the effects of IRI and in this study we tested baculovirus as a delivery vector for kidney cells. An *in vitro* ischaemia model was optimized in HEK293 cells using Antimycin A, which inhibits the mitochondrial electron transport, in combination with non-metabolizable 2-deoxyglucose. We constructed a recombinant baculovirus encoding Mnsod under the CMV promoter to deliver Mnsod into HEK293 and we investigated the effect of recombinant gene expression on IRI. We also investigated the effect of this virus on the innate and adaptive immune response of these cells. An RT² *Profiler* PCR Arrays study showed that the presence of Mnsod expression activated the innate immune response rather than the presence of the virus vector itself. The implications of these finding for the safe use of baculovirus in *in vivo* studies are discussed.

Poster / Viruses, Wednesday 16:00 **V24 STU**

Heterologous cell culture models for DWV

Nadine Möckel; Sebastian Gisder; Elke Genersch
Institute for Bee Research, Friedrich Engels-Straße 32, D-16540 Hohen Neuendorf, Germany
Address for correspondence: elke.genersch@rz.hu-berlin.de

Deformed wing virus (DWV) is a viral pathogen of the European honeybee (*Apis mellifera*). As typical for honeybee viruses, DWV normally causes covert infections. Only when transmitted by the ectoparasitic mite *Varroa destructor (V. destructor*) to pupae, DWV is able to cause overt infections characterized by adult bees emerging with malformed appendages, shortened abdomen and discolorations. To analyze the interaction between DWV and its target cells we recently established an *in vitro*-cell culture model for DWV using primary neuronal cells of honeybee pupae isolated from mushroom bodies. However, cell culture models based on primary cells isolated from pupae have several disadvantages like lack of reproducibility and limited (i.e. seasonal) availability. We recently published the establishment of a cell culture model for honey bee pathogenic *Nosema* spp. based on commercial permanent cell line derived from the gypsy moth (*Lymantria dispar*). Encouraged through this success, we now aimed at finding a lepidopteran cell line permissive for DWV in order to establish a heterologous cell culture model for DWV. Several lepidopteran cell lines proved to be susceptible to DWV infection although replicated efficiency varied between the cell lines. Successful infection with DWV could be demonstrated by DWV-specific

fluorescent-*in situ* hybridisation (FISH). Viral replication could be demonstrated by strand-specific *in situ* hybridisation using DIG labelled oligonucleotide probes. These cell culture models provide a novel means to analyze DWV-host cell-interactions and to develop new treatments against this pathogen

Poster / Viruses, Wednesday 16:00 **V25**

NTPase-like proteins from the banchine ichnovirus GfIV: transcriptional analysis, molecular modeling and functional assays

Michel Cusson[1,2]; Audrey Nisole[1,3]; Benoit Aspirault[2]; Mathieu Landry[2]; Catherine Béliveau[1]; Halim Maaroufi[3]
[1] Natural Resources Canada, Canadian Forest Service, Laurentian Forestry Centre, 1055 du P.E.P.S., Québec, QC, G1V 4C7, Canada; [2] Département de biochimie, de microbiologie et de bio-informatique, Université Laval, Québec, QC, G1V 0A6, Canada; [3] Institut de biologie intégrative et des systèmes, Université Laval, Québec, QC, G1V 0A6, Canada.
Address for correspondence: michel.cusson@nrcan.gc.ca

Many endoparasitic wasps inject a polydnavirus into their caterpillar hosts during oviposition. The viral entities associated with ichneumonid parasitoids are referred to as "ichnoviruses" (IV). Like other polydnaviruses, IVs have a segmented dsDNA genome and induce host physiological perturbations that benefit the immature wasp. IVs have so far been observed in two subfamilies, the Campopleginae and Banchinae. Most of what we know about banchine IVs is based on the characterization of a single virus, the *Glypta fumiferanae* ichnovirus (GfIV), whose virions and gene content differ considerably from those of the more extensively studied campoplegine IVs. One such difference is the presence of a gene family whose members are related to the D5 NTPases of large DNA viruses. The latter enzymes feature an N-terminal primase domain and a C-terminal NTPase/helicase domain. However, none of the nine GfIV NTPase-like proteins contain a detectable primase domain, and the NTPase/helicase domain, when present, has undergone substantial erosion. Here we report on the qPCR transcriptional analysis of GfIV genes following parasitization of pre-diapause 1st instar *Choristoneura fumiferana*. The most highly expressed gene is a member of the NTPase-like family, and its expression persists through diapause and post-diapause. Its coding sequence was cloned and the protein produced in a bacterial expression system. Although molecular modeling of the predicted protein suggested the presence of a functional ATP binding site, the recombinant protein displayed no ATPase activity. We are now examining whether it could serve as an ATP trap or form complexes with endogenous NTPases to inactivate them.

Poster / Viruses, Wednesday 16:00 **V26 STU**

Enhanced expression of the glycoproteins of Aujeszky's Disease Virus using a baculovirus expression system

Bit Na Rae Yun; Sung Min Bae; Jae Bang Choi; Jun Beom Lee; Hee Jung Kim; Soo Dong Woo
Department of Agricultural Biology, Chungbuk National University, Cheongju, 363-763, Korea
Address for correspondence: sdwoo@cbnu.ac.kr

Aujeszky's disease (AD), also called pseudorabies, is an infectious viral disease caused by an alpha herpes virus and has domestic and wild pigs, as well as a wide range of domestic and wild animals, as the natural host. Aujeszky's disease virus (ADV) virions contain several envelope glycoproteins. Among them, gB, gC and gD are regarded as the major immunogenicity proteins and the antibodies induced by them can neutralize virus in vitro or in vivo. In this study, we expressed these glycoproteins with various methods using the baculovirus expression vector system (BEVS) and compared expression level. Expression in two types of baculoviruses, *Autographa californica* nuclear polyhedrosis virus (AcNPV) and *Bombyx mori* nuclear polyhedrosis virus (BmNPV), resulted that the AcNPV system is superior to BmNPV system for the expression of ADV glycoproteins. To enhance the expression of recombinant proteins, we used a partial polyhedrin fusion system. Fusion expression of ADV glycoproteins with partial polyhedrin increased comparing native form of gB, gC and gD.

Poster / Viruses, Wednesday 16:00 **V27**

Transcriptomic analysis of virion protein genes of *Chilo iridescent virus*

Ikbal A. Ince[1,2,3], Monique M. van Oers[1] and Just M. Vlak[1].
[1]Laboratory of Virology, Wageningen University, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands, [2]Department of Biology, Faculty of Arts and Sciences, Giresun University, 28049 Giresun, Turkey, [3]Yeditepe University, Department of Genetics and Bioengineering, 34755 İstanbul Turkey
Address for correspondence: agah.ince@wur.nl

Chilo iridescent virus (CIV) is the prototype of the genus *Iridovirus.* Insect iridoviruses infect weevils and caterpillars that cause major problems in agro-ecosystems. CIV is a potential biocontrol agent of weevils in tea and hazelnut in the North-East of Turkey as well as in cotton in the South-Eastern and Mediterranean parts of this country. Iridoviruses have also been found to infect insects that transmit plant pathogens and/or parasites of medical importance, such as mosquitoes, whiteflies and grasshoppers. Fundamental knowledge of iridoviruses, an area so far underexplored, is important in view of their possible use as biological control agents for pest insects. Although data have been collected over the past years about the iridovirus infection cycle, many fundamental questions still remain to be answered concerning the structure and the nature of virus-host interactions, including the initial steps in virus infection such as the onset of transcription of viral genes. The complete genome sequence of CIV is known for ten years and the virion proteins have recently been determined (Ince *et al*., Virology 405, 253-258), but data on the transcriptome are limited. In this study the expression classes of the CIV virion proteins (i.e. immediate-early, delayed-early and late genes) were unraveled by combining drug treatments and RT-PCR studies. Interestingly, CIV virion protein gene transcripts belong to either the immediate-early or late gene classes. Genes encoding virion proteins may not only be important to build the structure of the virions, but may also play crucial roles in the initial stages of infection.

Cross Divisional Workshop (Microbial Control and Bacteria) Wednesday, 20:30-23:00

Industry Innovation in Biocontrol

Organizers: Kenneth E. Narva, Dow AgroSciences

Workshop Paper, Wednesday 20:30 **135**

Insect resistance management in the bag: pyramided traits and seed blend refuges

Nicholas P. Storer,
Dow AgroSciences LLC, 9330 Zionsville Rd, Indianapolis, IN, 46268, USA; nstorer@dow.com

The first insect-protected transgenic maize and cotton crops contained single Cry1A toxins from *Bacillus thuringiensis* Berliner (*Bt*). To slow the evolution of resistance in target insect populations, *Bt* crop growers have been required to plant separate portions of their fields to non-*Bt* varieties. These non-*Bt* patches serve as structured refuges for susceptible insects to develop and be available to mate with any resistant insects developing in the Bt fields. However, ensuring full grower adherence to structured refuge requirements has proven to be challenging. In recently introduced products, such as SmartStax® insect-protected maize,

multiple *Bt* proteins with different modes of action against each key target pest species are combined in individual plants. Simulation models indicate that the evolution of resistance to these pyramided-trait products is expected to be dramatically delayed, permitting significant reductions in the required refuge percentages. Reduced refuge size and redundant killing of pests has recently led the U.S. Environmental Protection Agency to allow the lepidopteran and coleopteran non-*Bt* refuge for SmartStax in the US Corn Belt to be provided as a seed blend. This product shifts the responsibility for refuge deployment from the grower to the trait provider, greatly simplifying farm management while ensuring the appropriate refuge is planted. These advances – pyramiding multiple *Bt* traits and blending refuge seed – reduce the uncertainties associated with resistance management for *Bt* crops and extend their expected durability.

Workshop Paper, Wednesday 20:45 **136**

Pyramided crops incorporating RNAi technology

William Moar; Tom Clark; Parthasarathy Ramaseshadri; Gerrit Segers; Graham Head.
Monsanto Company, 800 North Lindbergh, Creve Coeur, MO 63167
Address for correspondence: william.moar@monsanto.com

The expression of dsRNA in crop plants, such as maize, to control damaging insect pests such as western corn rootworm (WCR, *Diabrotica virgifera virgifera* LeConte) represents a new class of environmentally friendly insect control traits. As with any new plant-expressed insect control trait, the potential for WCR to evolve resistance to dsRNA needs to be considered. This presentation will discuss information regarding: pyramiding of WCR-active dsRNA with Cry3Bb1 and potential for cross resistance, determination of effective dose, and potential mechanisms of resistance to dsRNA.

Workshop Paper, Wednesday 21:00 **137**

Successes and challenges in commercialization of micro- and macro-biologicals

Andrew P. Brown[1]; Mark Howieson[2]; Julie Graesch[2]
[1]Becker Underwood Ltd., Harwood Road Industrial Estate, Harwood Road, Littlehampton, West Sussex, BN17 7AU, UK,
[2]Becker Underwood Inc., 801 Dayton Avenue, Ames IA 50010, USA.
Address for correspondence: andrew.brown@beckerunderwood.com

Becker Underwood produces and markets a number of micro- and macro-biologicals. Discovery to launch requires a systematic development process in order to have commercial success. First is isolation of species and strains. Because biological control generally is a strain-specific characteristic, this stage often results in the establishment of collections of thousands of cultures. Initial efficacy screening of cultures is first carried out by using *in vitro* or small scale controlled environment evaluations, although DNA techniques likely will become more widely adopted. Successful candidates emerging from initial screening are advanced to production and more advanced efficacy trials. These stages are time and resource intensive. Production of biological control agents is carried out by a number of different methods, including liquid fermentation of entomopathogenic nematodes [e.g. *Steinernema feltiae*; Nemasys®] and beneficial bacteria [e.g. *Bacillus subtilis*; Integral®] as well as solid-state production of entomopathogenic fungi [e.g. *Metarhizium anisopliae*; Green Guard®] and disease-controlling fungi [e.g. *Trichoderma harzianum*; Tricho Plus®]). Production research typically focuses on methods required to produce a certain life-stage of the organism, for example, fungal/bacterial spores or infective juvenile of entomopathogenic nematodes, at economically viable yields. Successful candidates are then formulated to maximize efficacy, stability, and to improve ease of use. Formulated end-use products are then entered into an extensive trial program to gain understanding of how it will perform in a crop system and support regulatory registration requirements.

Workshop Paper, Wednesday 21:15 **138**

Novel targeted delivery systems in biocontrol

Nick Jessop
Exosect Ltd, Leylands Business Park, Colden Common, Winchester, SO21 1TH, UK

Workshop Paper, Wednesday 21:30 **139**

RNAi products platform for invertebrates' health and Targeted Pest Control

Ben-Chanoch Eyal., Glick Eitan, Paldi Nitzan, Yarden Gal
Beeologics Inc. 11800 SW 77th Avenue, Miami, Florida 33156 Tel: +1 305-233-6564, www.beeologics.com
Address for correspondence: eyal@beeologics.com

Ribonucleic Acid interference (RNAi) applications for invertebrates are on the scientific agenda since RNAi was initially introduced. The work done on c-elegance not only was awarded with the Nobel Prize in 2006, but also triggered many initiatives that focused on how the RNAi mechanism can be used for pest management. RNAi based products have been developed by Beeologics to control honeybee viruses and parasites. The initial product to be introduced is Remebee® – an anti-viral agent fed to the bees protecting them from acute infection caused by the Israeli Acute Paralysis Virus (IAPV). This virus has been proved to cause honeybee mortality and identified as one of the participating factors of Colony Collapse Disorder (CCD). The technology platform built in the creation of Remebee covers all aspect of introduction of a new product. It specifically includes the methods and automation required to produce large amounts of consistent quality RNAi; a major need in the effort of bringing a product to market. The capability of making large and inexpensive RNAi materials, helped in shaping innovative and accelerated development processes. This platform is now supporting the development of several complementary RNAi products for the honeybees as well as a variety of application for targeted pest control.

Workshop Paper, Wednesday 21:45 **140**

Integration of Entomopathogenic Fungi into Insecticide Resistance Management Programs for Control of Sucking Insect Pests

Jarrod Leland
Novozymes Biologicals, Inc., 5400 Corporate Circle, Salem VA 24153 United States
Address for correspondence: jrrl@novozymes.com

Insecticide resistance is increasing globally for sucking insect pests. In many regions growers are finding limited options to control pests such as thrips, whiteflies, and plant bugs to name a few. Several factors have contributed to increased resistance and significance of sucking insect pests. New insecticide actives are slower to reach the market due to increased regulatory barriers. Intensified agricultural production had increased pressure on insecticides. The use of transgenic Bt row crops has reduced the application of chemical insecticides targeted for Lepidoptera that coincidentally controlled sucking insect pests. Among the options for microbial biocontrol, fungi may provide the best option due to their contact mode of action. This presentation will describe several examples in row crops and high value crops were there is a role for entomopathogenic fungi in insecticide resistance

management programs and what advances have been made or are needed to fill that gap.

Workshop Paper, Wednesday 22:00 **141**

Novel microbial controls from the Enterobacteriaceae

Mark Hurst[1] and Trevor Jackson[1]
[1]Biocontrol & Biosecurity, AgResearch, Lincoln Research Centre, Private Bag 4749 Christchurch, 8140, New Zealand.
Address for correspondence: mark.hurst@agresearch.co.nz

The bacteria *Serratia entomophila* and *Yersinia entomophaga* have both been isolated from larvae of the New Zealand grass grub, *Costelytra zealandica* (Coleoptera: Scarabaeidae), from New Zealand soils. *Serratia entomophila* has been used successfully as a biocontrol agent against *C. zealandica* and has been applied in both liquid and granule formulations applied into the soil using a seed drill. *Serratia entomophila* is only infective for *C. zealandica* larvae, while another Gram-negative bacterium, *Y. entomophaga*, shows activity towards a number of coleopteran and lepidopteran pests, with a high activity against members of the Scarabaeidae where LD50s of approximately 5x 10^4 bacteria/larva have been determined. Unlike the chronic disease induced by *S. entomophila*, *Y entomophaga* typically causes death within 2-5 days of infection. Both *S. entomophila and Y. entomophaga* can be economically fermented in large volumes producing >1 x 10^{10} bacteria /mL within 24 hours. Formulated *Y. entomophaga* has shown significant field control when applied as a foliar spray against chrysomelid beetles (*Eucolaspis* sp) or in bait formulation against ground dwelling lepidoptera (*Wiseana* sp.). In pot trials *Y. entomophaga* has shown comparable efficacy to Spinosad or Dipel against diamond back moth (*Plutella xyllostela*). It is envisaged that technologies used to formulate *S. entomophila* will be applicable to *Y. entomophaga* allowing the development of an economically viable products for microbial control.

THURSDAY – 11 August

Contributed Papers

Thursday, 8:30-9:45

Fungi 2

Contributed Paper, Thursday 8:30 **142**

Natural occurrence and artificial establishment in *Pinus radiata* seeds and roots with *Beauveria bassiana*

Travis R Glare[1]; Stephen D. Reay[2]; Michael Brownbridge[3], Aimee C. McKinnon[1], Tracey L. Nelson[4]

[1]Bio-protection Research Centre, PO Box 84, Lincoln University, Lincoln 7647, New Zealand, [2]Silver Bullet Forest Research, Auckland, New Zealand, [3]Vineland Research and Innovation Centre, 4890 Victoria Ave. N., Box 4000, Vineland Station, Ontario, Canada L0R 2E0, [4]AgResearch Ltd., Lincoln Research Centre, Private Bag 4749, Christchurch 8140, New Zealand

Address for correspondence: travis.glare@lincoln.ac.nz

The fungus *Beauveria bassiana* is known to be a naturally occurring endophyte in a range of plant species, including pine trees, *Pinus* spp. *Pinus radiata* is the predominant species grown in New Zealand's plantation forests. As part of a study to investigate *B. bassiana* as a biocontrol agent of bark beetle pests of pine, we have identified *Beauveria* as endophytes in mature pines in forests across New Zealand. We investigated if *B. bassiana* isolates recovered from mature pines could be inoculated onto seed and roots, and subsequently establish as endophytes in the seedlings. Using culturing methods, it was found that both seed coating and root dipping resulted in establishment in the seedlings, however the fungus did not persist past nine months except in one seedling. Culturing methods for identifying endophytic *Beauveria* may not fully recover all fungi. Consequently, we developed PCR-based specific primers for *Beauveria*. Using molecular identification methods, analysis of seed and seedlings derived for one source of seed in New Zealand showed very high levels of *Beauveria* occurring as endophytes, suggesting that *Beauveria* may be vertically maintained in pines. Furthermore, more than one genotype was recovered from some single seedlings.

Contributed Paper, Thursday 8:45 **143**

Recent studies to increase the thermotolerance of entomopathogenic fungi

Jae Su Kim[1,2]; Margaret Skinner[1]; Svetlana Gouli[1]; Jae Young Choi[3]; Yeon Ho Je[3]; & Bruce L. Parker[1]

[1]Entomology Research Laboratory, University of Vermont, 661 Spear Street, Burlington, VT 05405-0105, USA, [2]Research Institute for Agriculture and Life Sciences, Seoul National University, Seoul 151-742, Republic of Korea, [3]Department of Agricultural Biotechnology, College of Agriculture & Life Sciences, Seoul National University, Seoul 151-742, Republic of Korea.

Address for correspondence: bparker@uvm.edu

For successful commercialization of entomopathogenic fungi a problem of low thermotolerance must be overcome. To increase thermotolerance the effects of nutritional manipulation of culture media and oil-coating of conidia of *Beauveria bassiana* and *Metarhizium anisopliae* isolates were investigated, followed by pairing of similar isolates. Millet grain and whey permeate-based cultures produced more thermotolerant conidia than ¼SDAY cultures. Conidia produced on millet or whey permeate were more hydrophobic and exhibited greater thermotolerance than those produced on ¼SDAY. The mycotized millet grains were further coated with plant oils. Conidia with a corn oil coating were superior to sunflower- and cotton seed oil coatings in thermotolerance. Pairing of two *B. bassiana* isolates was done to generate new colonies with enhanced thermotolerance by possible hyphal fusion. One colony with completely different morphology was isolated from the paired culture and it had the highest thermotolerance without significant loss of virulence. These results suggest that the thermotolerance of entomopathogenic fungi can be enhanced by media-manipulation, pairing and oil-coating of conidia.

Contributed Paper, Thursday 9:00 **144**

Optimization of a media with antimicrobial effects on the germination of *Beauveria bassiana*

David Ruelas-Ayala, Cipriano Garcia-Gutierrez

CIIDIR-COFAA IPN Sinaloa. Department of Biotechnology. Blvd. Juan de Dios Batiz Paredes No. 250 AP. 280 Guasave, Sinaloa, Mexico. CP. 8110. Tel and Fax: (52) 687 872 9626 or (52) 687 625 8729.

Address for correspondence: cgarciag@ipn.mx

A selective media is required to obtain pure isolates of *B. bassiana* from soil, so the aim of this work was to evaluate the effect of different antibiotics on the growth and germination. Media containing copper chloride (100, 200, 300 and 400 ppm), crystal violet (1, 2, 3 and 4 ppm) and sodium benzoate (300, 400, 500 and 600 ppm) were used in triplicate using cages with agar-based medium and yeast extract. Copper chloride caused no significant effect on the spore germination (90%), and the growth at 0-300 ppm (Tukey 0.01), however to 400 ppm there was significant differences, decrease in growth. The crystal violet has a significant decrease of germination (55%) above 1 ppm remained without significant differences at 1 to 4 ppm. Sodium benzoate above 300 ppm reduced 50% the germination, although the concentration increases the germination rate decreases until a very low germination of 17.66% at 600 ppm, however growth was not affected by the concentrations of sodium benzoate, once germinated spore fungus continues to grow without any problem. Antibiotics at 200 ppm of copper chloride, crystal violet 4 ppm and 300 ppm of sodium benzoate, can be used to obtain a selective media for this fungi.

Contributed Paper, Thursday 9:15 **145**

Rhizosphere competens of insect pathogenic fungi in the control of *Othiorhynchus sulcatus* in strawberries under cold climatic conditions

Ingeborg Klingen[1], Karin Westrum[1]

Norwegian Institute for Agricultural and Environmental Research (Bioforsk), Plant Health and Plant Protection Division, Høgskoleveien 7, N-1432 Ås, Norway.

Address for correspondence: ingeborg.klingen@bioforsk.no

The vine weevil, *Othiorynchus sulcatus*, is a serious pest in strawberries in Norway and biological control methods are needed to combat this pest. In this study, the rhizosphere competence of two cold active Norwegian fungal isolates *(Metarhizium anisopliae* isolate NCRI 250/02 and *Beauveria bassiana* NCRI 12/96) and the well known Ma43 originating from Austria were tested. This was done by estimating fungal concentrations in the bulk and rhizosphere soil surrounding the strawberry plant roots by counting colony forming unists (CFUs). The highest numbers of *B. bassiana* NCRI 12/96 CFUs were seen in the rhizosphere at $1.87x10^9$ per liter soil 3 months after application. The highest numbers of *M. anisopliae* NCRI 250/02 CFUs were seen in the rhizosphere at $2.41x10^9$ per liter soil 1 year after application. Numbers of CFUs for the *M. ansiopliae* Ma43 CFUs were generally lower than for the Norwegian isolates, but also for this isolate a higher fungal concentration was found in the rihzosphere soil than in the bulk soil.

Contributed Paper, Thursday 9:30 **146**

Evaluation of *Fusarium coccophilum* as a biological control option for armoured scale insects

Nicola A. Mauchline[1]; Garry Hill[1]
[1]The New Zealand Institute for Plant & Food Research Limited (Plant & Food Research), 412 No. 1 Road, RD 2, Te Puke, New Zealand.
Address for correspondence: nicola.mauchline@plantandfood.co.nz

Biological control of armoured scale insects offers advantages over insecticides including host specificity, reduced threat to non-target organisms, decreased risk of resistance development and phytoxicity, and enhanced compatibility with other biological control agents. The fungus, *Fusarium coccophilum* ((Desm.)Wollenw. & Reink), is a highly virulent obligate parasite of armoured scale insects. Twenty one New Zealand isolates demonstrated efficacy against *Hemiberlesia lataniae* (Signoret). Under laboratory conditions (20°C ± 2°C, 90% RH ± 10%), insect mortality ranged from 50-100%, with mycosis resulting 7-21 days after inoculation. An LT_{50} of 7-8 days was observed for two virulent isolates, with a flat response to increasing inoculum concentration. A 97% reduction in the number of scale insect crawlers produced and up to75% insect mortality resulted when scale infested *Actinidia deliciosa* ((A. Chev.) C.F. Laing & A.R. Fergusson 'Hayward') vines were sprayed with an aqueous formulation ($4x10^6$ conidia/mL). No phytoxicity was observed. Laboratory trials indicated that *F. coccophilum* host range was restricted to the family Diaspididae, where mycosis was observed within the genera *Hemiberlesia, Aspidiotus, Quadraspidiotus* and *Aonideiella*. No mortality was observed for non-target organisms, honey-bee (*Apis mellifera*), longtailed mealy bug (*Pseudococcus longispinus*), and brown headed leafroller (*Ctenopseustis obliquana*). The attributes demonstrated by *F. coccophilum* indicate suitability for use as a biological control agent for armoured scale insects.

Contributed Papers Thursday, 8:00-9:45

Virus 5

Contributed Paper, Thursday 8:00 **147 STU**

Immune-gene responses in *Penaeus monodon* shrimp following infection with *Gill-associated virus*

Darren J. Underwood[1]; Melony J. Sellars[2]; Jeff A. Cowley[3]; Karyn N. Johnson[1]
[1]School of Biological Sciences, The University of Queensland, St. Lucia, Qld 4072, Australia, [2]CSIRO Food Futures National Research Flagship, CSIRO Livestock Industries, Queensland Biosciences Precinct, St. Lucia, Qld 4067, Australia, [3]CSIRO Food Futures National Research Flagship, CSIRO Marine and Atmospheric Research, Dutton Park, Qld 4102, Australia.
Address for correspondence: karynj@uq.edu.au

Over the past 30-40 years the production of cultured shrimp has increased exponentially to rival the volume of wild-caught shrimp. With the increase in production volume and economic value the damage caused by viral diseases has sparked an increase in research into shrimp antiviral responses. Some components of the shrimp antiviral response have been elucidated: Binding molecules such as small GTPases, humoral interactions such as Toll, prophenoloxidase and lectin pathway components and cellular responses such as the Jak-STAT and RNAi pathways. To date, data on pathogen defence responses has been gathered almost exclusively from studies of *White spot syndrome virus* (WSSV). However, to understand the mechanisms underlying shrimp antiviral immunity more comprehensively, examining responses induced by other viruses may prove important. In Australia, *Gill-associated virus* (GAV), a virus closely related to *Yellow-head virus* (YHV), is prevalent and frequently causes disease and mortalities in farmed *Penaeus monodon* shrimp. GAV and WSSV are diverse viruses: GAV has a 26.2 kb (+) ssRNA genome and belongs to the *Roniviridae* family, whereas WSSV has a ~ 300 kb dsDNA genome and belongs to the *Nimaviridae* family. We have analysed the expression of several genes in response to GAV that have been shown to be involved in the shrimp response to other viruses, including WSSV. GTPases and lectins were selected for analysis as immune components that are thought to have roles in pathogen recognition. We present data showing that in some but not all cases these genes are differentially regulated in response to GAV.

Contributed Paper, Thursday 8:15 **148**

Subcellular localization and functional characterization of MdBV IκB - like protein N5 in *Drosophila* mbn2 cells

Kavita Bitra[1]; Richard Suderman[1]; Michael R. Strand[1]
Department of Entomology, University of Georgia, 120 Cedar street, Athens, Georgia - 30602, USA.
Address for correspondence: kbitra@uga.edu

Viruses in the family polydnaviridae are all symbionts of parasitoid wasps whose hosts are primarily larval stage of Lepidoptera. A key function of polydnaviruses is suppression of host immune defenses, which allows the offspring of associated parasitoids to successfully develop. NF- κB transcription factors are components of Toll and Imd pathways that regulate a diversity of immune functions in insects. In turn, the encapsidated genome of *Microplitis demolitor* bracovirus (MdBV) encodes multiple ankyrin repeat genes, which resemble known negative regulators of NF-κBs called inhibitory κBs (IκBs). Here we report that MdBV Ank- H4 and –N5 proteins directly bind insect NF- κBs. We further showed that Ank- N5 mostly localized to the nuclear fractions and can suppress the endogenous expression of AMP genes mediated by Toll and Imd pathways.

Contributed Paper, Thursday 8:30 **149**

Studies of the subgenomic RNA3 and protein B2 of Wuhan Nodavirus

Xi Zhou[1]; Yang Qiu[1]; Jiamin Zhang[1]; Congyi Zheng[1]; Yuanyang Hu[1]
State Key Laboratory of Virology, College of Life Sciences, Wuhan University, Wuhan, Hubei 430072 China
Address for correspondence: zhouxi-whu@139.com

Nodaviruses are small nonenveloped spherical viruses with a bipartite genome of RNAs. Wuhan Nodavirus (WhNV) is a newly identified from Pieris rapae (P. rapae) larva in Wuhan, China. In nodaviruses, subgenomic RNA3 (sgRNA3) plays a critical role in viral replication and survival, as it coordinates the replication of two viral genomic RNAs (RNA1 and RNA2) and encodes for protein B2, which is a potent RNA-silencing inhibitor. Despite of its importance, the molecular mechanisms of WhNV sgRNA3 synthesis and how WhNV B2 protein function as an RNAi suppressor remained unclear. Here, we uncovered that sgRNA3 of *Wuhan nodavirus* (WhNV) is internally initiated from a promoter on the negative template of genomic RNA1, and both the secondary structure and the primary sequence of WhNV sgRNA3 are required for promoter activity. Moreover, we found that WhNV sgRNA3 synthesis is antagonized by the replication of WhNV genomic RNA2, which encodes a viral capsid precursor protein, and this sgRNA3 synthesis is also able to trans-activate the RNA2 replication. Our further study on WhNV B2, which is encoded by sgRNA3, revealed that WhNV B2 blocks RNAi by the mechanism of dsRNA and siRNA sequestration, and both the primary RNA-binding domain and the homodimerization domain of WhNV B2 are required for WhNV B2 to suppress RNAi-suppression. These findings indicated novel models for WhNV sgRNA3 synthesis and the WhNV B2-mediated RNAi suppression.

Contributed Paper, Thursday 8:45 **150**

Gloverin: an antiviral protein induced in *Trichoplusia ni* during baculovirus infection

Eric J. Haas-Stapleton; Daniela Moreno-Hable; Angelica Dulce
Department of Biological Sciences, California State University Long Beach, 1250 Bellflower Blvd., Long Beach, CA 90840 USA.
Address for correspondence: ehaas@csulb.edu

Antimicrobial peptides are generated in insects exposed to pathogens for combating infection. Gloverin is a small bacteriostatic peptide that is induced in the hemocytes of *Trichoplusia ni* larvae exposed to bacteria. Gloverin is expressed as a propeptide that is hypothesized to perforate or inhibit the formation of the bacteria cell wall. We show that gloverin is also induced in *T. ni* infected with the baculovirus *Autographa californica* M nucleopolyhedrovirus (*Ac*MNPV). To determine the role of gloverin during *Ac*MNPV infection, we cloned pro- and mature gloverin from total RNA isolated from the hemocytes of *T. ni*, incorporated His- and V5-tags, expressed gloverin in Sf9 cells and purified the peptide using nickel-affinity chromatography and dialysis. Using SDS-PAGE, the sizes of purified pro- and mature gloverin were 22 and 19 kDa, respectively. To evaluate the antiviral properties of gloverin, we incubated *Ac*MNPV budded virus (BV) in purified pro- or mature gloverin and conducted plaque assays using Sf9 cells to quantify changes in BV infectivity. The infectivity of BV incubated in the presence of mature and pro-gloverin was reduced 75 % and 92 %, respectively, relative to vehicle controls. The results suggest that gloverin may display broad antimicrobial activity.

Contributed Paper, Thursday 9:00 **151**

The impact of Dicer-2 and *Wolbachia* on antiviral protection in *Drosophila*

Lauren M. Hedges, Ryuichi Yamada, Scott L. O'Neill and Karyn N. Johnson
School of Biological Sciences, The University of Queensland, Brisbane, Australia
Address for correspondence: karynj@uq.edu.au

Modes of antiviral protection in *Drosophila* are diverse and include both host and non-host factors. The siRNA pathway is a key defense, which inhibits viral replication by sequence specific degradation of the viral RNAs. In *Drosophila*, antiviral protection is also mediated by the bacterium *Wolbachia*, however the mechanism of this protection is not known. When infected with RNA viruses *Drosophila C virus* (DCV- *Dicistroviridae*) or *Flock House virus* (FHV - *Nodaviridae*), *Wolbachia*-infected flies have delayed virus-induced mortality. In contrast there is no *Wolbachia*-mediated protection against the DNA virus *Insect Iridescent Virus 6*. To determine whether *Wolbachia*-mediated protection is dependent on the siRNA pathway, flies with mutations in the siRNA pathway components Dicer-2, r2d2 and Ago2 were challenged with either FHV or DCV. Compared to *Wolbachia*-free flies, DCV and FHV-induced mortality was delayed in both r2d2 and Ago2 mutant flies infected with *Wolbachia*. *Wolbachia*-mediated protection was also observed in Dicer-2 mutant flies when infected with FHV, indicating that *Wolbachia*-mediated protection is independent of the canonical siRNA pathway. Unexpectedly, in *Wolbachia*-free flies, Dicer-2 mutants had delayed virus-induced mortality compared to wild-type *Drosophila* when infected with DCV, but not when infected with FHV. We confirmed that loss of Dicer-2 was protective to *Drosophila* infected with DCV but not FHV, using fllies that endogenously express hairpin RNAs which target Dicer-2. Since the loss of the other siRNA pathway components had a deleterious effect on fly survival, we hypothesise that the protective effect of loss of Dicer-2 during DCV infection is due to a function of Dicer-2 that doesn't involve the siRNA pathway.

Contributed Paper, Thursday 9:15 **152**

Apoptosis in host defense and productive infection in *Amsacta moorei entomopoxvirus* infected cells

Srini Perera[1,2]; Peter Krell[2]; Basil Arif[1]
[1]Laboratory for Molecular Virology, GLFC, Sault Ste Marie, ON, Canada, [2]University of Guelph, ON, Canada.
Address for correspondence: barif@nrcan.gc.ca

The CF-70-B2 cells derived from the spruce budworm (*Choristoneura fumiferana*) undergo apoptotic cell death when infected with *Amsacta moorei entomopoxvirus* (AMEV); as characterized by membrane blebbing, formation of apoptotic bodies and induction of caspase 3/7 activity. The apoptotic response was reduced significantly when infected with UV-inactivated AMEV, but not when infected in the presence of DNA synthesis inhibitor, cytosine β-D-arabinofuranoside. Hence, only the pre-replicative events of AMEV infection were involved in apoptosis induction in CF-70-B2 cells, at the early stages. The virus eventually overcomes this host antiviral response and replicates to high virus titers accompanied by high levels of caspase 3/7 activity, which suggested involvement of caspases at late infection stages. The CF-70-B2 cells were less permissive to infection in comparison to LD-652 cells (a *Lymantria dispar* cell line routinely used for propagation of AMEV) in terms of both budded and occluded virus production. At late infection stages, the highly permissive LD-652 cells also showed characteristics of apoptosis such as DNA fragmentation, nuclear condensation and increased caspase-3/7 activity. Induction of apoptosis in LD-652 cells was dependent on viral DNA replication and/or late gene expression. When CF-70-B2 and LD-652 were treated with the general caspase inhibitor Z-VAD-FMK, infected cells were more resistant to cell lysis and the virus titers were greatly reduced. Our results suggest that AMEV induced late-apoptosis plays a crucial role in productive virus infection.

Contributed Paper, Thursday 9:30 **153**

Understanding the roles of p53 and the DNA damage response in baculovirus replication and baculovirus-induced apoptosis

Ning Huang[1], Wenbi Wu[1], A. Lorena Passarelli[1], George F. Rohrmann[2], and Rollie J. Clem[1]
[1]Division of Biology, Kansas State University, Manhattan, KS; [2]Department of Microbiology, Oregon State University, Corvallis, OR, USA
Address for correspondence: rclem@ksu.edu

The p53 protein is involved in apoptosis and cell cycle arrest in mammals, and thus inactivation of p53 is important for replication of some DNA viruses. *Drosophila* encodes a single p53 ortholog which is involved in apoptosis stimulated by DNA damage, but not in DNA damage-induced cell cycle arrest. A previous report identified an interaction between human p53 and the sulfhydryl oxidase protein p33 (Ac92) from *Autographa californica* M nucleopolyhedrovirus (AcMNPV), but it is unclear whether a similar interaction occurs with p53 from baculovirus host cells, and whether p53 has any involvement in baculovirus replication or baculovirus-induced apoptosis. Orthologs of p53 were identified in *Spodoptera frugiperda* (Sfp53) and *Bombyx mori*. Sfp53 interacted with Ac92 by co-immunoprecipitation, similar to human p53, and overexpression of Sfp53 induced apoptosis in Sf9 cells. Levels of endogenous Sfp53 protein increased dramatically after treatment with DNA damaging agents or AcMNPV infection, and this increase in Sfp53 protein levels was blocked by caffeine, an inhibitor of the DNA damage response. Caffeine also caused delayed and reduced viral late gene expression, suggesting that AcMNPV may utilize enzymes and factors involved in DNA repair to achieve full levels of replication. However, silencing of Sfp53 expression by RNAi (as verified by immunoblot) had no apparent effect on apoptosis induced by infection with a p35 mutant strain of AcMNPV. These results suggest that a DNA damage response

is triggered by AcMNPV infection, but one of the major apoptotic effectors of the response, Sfp53, is not required for AcMNPV-induced apoptosis.

Contributed Papers Thursday, 8:15-10:00

Diseases of Beneficial Invertebrate 3

Contributed Paper, Thursday 8:15 **155 STU**

Susceptibility to viral infection and pathogenicity of White Spot Disease (WSD) in non-model crustacean host taxa from temperate regions

Kelly S. Bateman and Grant D. Stentiford
European Union Reference Laboratory for Crustacean Diseases, Centre for Environment, Fisheries and Aquaculture Science (Cefas), Barrack Road, The Nothe, Weymouth, Dorset, DT4 8UB, UK.
Address for correspondence: kelly.bateman@cefas.co.uk

Despite almost two decades since its discovery, White Spot Disease (WSD) is still considered the most significant known pathogen impacting the sustainability and growth of the global penaeid shrimp farming industry. Although most commonly associated with penaeid shrimp farmed in warm waters, the virus is also able to infect, cause disease and kill a wide range of other decapod crustaceans from temperate regions, including lobsters, crabs, crayfish and shrimp. Using principles laid down by the European Food Safety Authority (EFSA) we used an array of diagnostic approaches to provide a definitive statement on the susceptibility to White Spot Syndrome Virus (WSSV) infection in seven ecologically or economically important European crustacean species. We chose four marine species: *Cancer pagurus*, *Homarus gammarus*, *Nephrops norvegicus and Carcinus maenas*; one estuarine species, *Eriocheir sinensis* and two freshwater species, *Austropotamobius pallipes* and *Pacifastacus leniusculus*. Exposure trials based upon natural (feeding) and artificial (intra-muscular injection) routes of exposure to WSSV revealed universal susceptibility to WSSV infection in these hosts, but also that relative susceptibility varied significantly between species. We describe the pathogenesis of WSD in these hosts and compare this to the well documented disease progression profile of model penaeid shrimp hosts.

Contributed Paper, Thursday 8:30 **156**

Behavior influences viral disease dynamics in the Caribbean spiny lobster

Donald C. Behringer[1]; Mark J. Butler[2]; Thomas W. Dolan[3]; Jeffrey D. Shields[3]; Jessica Moss[3]
[1]University of Florida, School of Forest Resources and Conservation & Emerging Pathogens Institute, Gainesville, FL 32653 USA, [2]Old Dominion University, Department of Biological Sciences, Norfolk, VA 23529 USA, [3]Virginia Institute of Marine Science, Gloucester Point, VA 23062 USA
Address for correspondence: behringer@ufl.edu

The spiny lobster *Panulirus argus* Latrielle supports the most valuable fishery in the Caribbean and plays an important ecological role in hard-bottom and coral reef communities. *Panulirus argus* is subject to a pathogenic virus (PaV1) that kills a fourth of the juvenile lobsters in Florida annually. We use the lobster-PaV1 disease dynamics to explore the role that host behavior and habitat structure play in altering the spread of disease, which is particularly relevant in social species such as spiny lobsters. Juvenile lobsters in Florida Bay are dependent on sponges for shelter, so following a large-scale sponge die-off they were crowded into the few shelters that remained. The PaV1 virus is spread by contact, so we anticipated this exaggerated aggregation would cause a spike in prevalence. Using laboratory experiments, field studies, and simulation modeling we capitalized on this "natural experiment" and discovered that host behavior can thwart the spread of disease, even in the face of habitat degradation. Moreover, we hypothesize that such mechanisms are so efficient in reducing disease transmission that local persistence of the pathogen depends on additional sources of disease, perhaps including infected post-larvae, which we recently discovered.

Contributed Paper, Thursday 8:45 **157 STU**

Pathogenesis of early *Hematodinium* sp. infection in Atlantic Canadian Snow crabs (*Chionoecetes opilio*)

Melanie A. Buote[1,2]; Richard J. Cawthorn[1,2]; Andrea L. Battison[1]
[1]AVC Lobster Science Centre, [2]Department of Pathology and Microbiology, Atlantic Veterinary College, University of Prince Edward Island, 550 University Avenue, Charlottetown, PE C1A 4P3 Canada
Address for correspondence: mabuote@upei.ca

Bitter crab disease (BCD) is a fatal disease of crustaceans caused by parasitic dinoflagellates of the genus *Hematodinium*. Infection with *Hematodinium* spp. is distributed worldwide in more than forty crustacean species, including several commercially important hosts. In Atlantic Canadian snow crabs (*Chionoecetes opilio*), BCD is generally a disease of juvenile males and immature females; infection is currently thought to be fatal. Late stages of BCD have been described in several hosts, characterized by marked parasitemia and multisystemic interstitial tissue parasitic infiltration. Early stages of natural infection have thus far not been fully investigated. We examined 160 snow crabs collected in the northern coastal bays of Newfoundland (4 sites) in fall 2010. Dinoflagellate infection level was determined via histologic examination of a wide range of tissues, including hepatopancreas, gonad, eyestalk, heart, gill, midgut, leg, and abdomen. Gross carapace examination revealed relatively low prevalence of macroscopic BCD (~4%). In contrast, microscopic infection levels of up to 100% were observed in selected collection sites. Preliminary infection distribution results from a subset of infected crabs suggest that early infections may have a predilection for hepatopancreas, gonad, and eyestalk. If infection always leads to disease, these high infection levels suggest that BCD outbreaks are imminent in snow crab populations at our collection sites. Alternatively, high levels of light infection may suggest that *Hematodinium* spp. are opportunistic pathogens and infection may only progress to BCD under certain environmental and/or host conditions which are currently unknown.

Contributed Paper, Thursday 9:00 **158**

***In vitro* cultivation of *Hematodinium* sp. and characterization of developmental stages**

Peter H. Gaudet[1,2]; Richard Cawthorn[1,2]; Spencer Greenwood[1,3]; Dorota Wadowska[4] ; Glenda Wright[3]
[1]AVC Lobster Science Centre, [2]Department of Pathology and Microbiology,[3]Department of Biomedical Sciences,[4]Electron Microscopy, Atlantic Veterinary College, University of Prince Edward Island, 550 University Ave. Charlottetown, PEI C1A 4P3 Canada.
Address for correspondence: phgaudet@upei.ca

Hematodinium sp. is a parasitic dinoflagellate of economically important crustaceans that was first reported off France in 1931 and has now spread globally. Infected crustaceans only display gross pathological changes (such as milky hemolymph and cuticle discolouration) during late stages of infection. *Hematodinium* sp. causes severe threats to crustacean populations as evidenced by numerous outbreaks in coastal waters: in *Callinectes sapidus* (eastern U.S.); *Nephrops norvegicus*, *Cancer pagurus*, and *Necora puber* (Europe); *Chionoecetes bairdi* (Alaska); and *Chionoecetes opilio* (Newfoundland and Labrador). Although knowledge of *Hematodinium*'s life history and survival strategies could benefit crustacean fisheries and further our academic understanding, few

successful attempts have been made to isolate and culture the parasite *in vitro* and to follow its subsequent development. Hemolymph samples collected from grossly infected *C. opilio* crabs from Newfoundland and Nova Scotia were placed in different *in vitro* culture media and processed for transmission electron microscopy upon arrival. Cultures were monitored to observe developmental transitions and further samples were processed for transmission and scanning electron, and light microscopy. Early sporont stages were present in infected hemolymph, which in culture gave rise to novel multinucleate forms that ultimately transitioned into dinospores. The dinospores have remained viable for approximately three months, suggesting that they may survive outside the host for extended periods of time, if sufficient nutrients exist. Our preliminary findings suggest novel life stages of the parasite, and that *Hematodinium* sp. has greater robustness than previously thought.

Contributed Paper, Thursday 9:15 **159 STU**

Trichocyst development and their potential role in *Hematodinium* sp. survival

Peter H. Gaudet[1,2]; Richard Cawthorn[1,2]; Spencer Greenwood[1,3]; Dorota Wadowska[4] ; Glenda Wright[3]
[1]AVC Lobster Science Centre, [2]Department of Pathology and Microbiology,[3]Department of Biomedical Sciences,[4]Electron Microscopy, Atlantic Veterinary College, University of Prince Edward Island, 550 University Ave. Charlottetown, PEI C1A 4P3 Canada.
Address for correspondence: phgaudet@upei.ca

Hematodinium sp. is a parasitic dinoflagellate of numerous crustaceans worldwide. The parasite causes Bitter Crab Disease, a lethal infection whereby the crab's hemolymph turns white, its meat becomes bitter, and eventually causes death due to respiratory and other organ dysfunction. Little is known of the basic biology of the parasite, including host-pathogen and pathogen-environment interactions. *Hematodinium* sp. possesses rod-like extrusomes (called trichocysts) which are hypothesized to serve predatory or defensive roles. Appearance of trichocysts marks transition of *Hematodinium* sp. from a trophont to sporont, and typically precedes *Hematodinium* sp. release from the host. Trichocysts begin as large Golgi-associated vesicles, and are eventually transported to their destination within the parasite, where they mature and attach to the cell membrane. A proteinaceous crystalline core forms and the vesicular membrane condenses around the core, forming very dense rods in the final stages. Our observations indicate that trichocyst development is not synchronized; mature and developing trichocysts may exist in a cell concurrently. Mature trichocysts, defined by membrane condensation, were observed prior to dinospore formation; however, development of some trichocysts in dinospores cannot be excluded. Furthermore, trichocysts often arrange in clusters, and may extrude as a net-like group. Our preliminary findings provide new information of trichocyst formation and function in *Hematodinium* sp., and may aid in our understanding of potential survival mechanisms outside the host.

Contributed Paper, Thursday 9:30 **160**

Parasites and diseases in marine copepods: challenges for future mass-production of live feed for fish larva production

Alf Skovgaard[1]
[1] University of Copenhagen, Faculty of Life Sciences, Department of Veterinary Disease Biology, Laboratory of Aquatic Pathobiology, Stigbøjlen 7, DK-1870 Frederiksberg C, Denmark
Address for correspondence: alf@life.ku.dk

Copepods are the natural food for many marine fish larvae, and the use of cultured copepods as life feed is, therefore, becoming increasingly important as more marine fish species are being produced in aquaculture. Large-scale cultivation of copepods may be challenged by diseases and parasites. In nature, marine copepods are hosts for parasitic organisms of many different taxonomic groups, including e.g. dinoflagellates, ciliates, paramyxans, nematodes and even other crustaceans. In addition, several parasites of copepods have yet not been investigated in relation to their taxonomic affiliation. The effects of parasites on their individual copepod host appear as diverse as the nature of the parasites. Some parasites (e.g. *Syndinium*) are lethal to their hosts and some (e.g. *Blastodinium*, *Paradinium*, and *Ellobiopsis*) are parasitic castrators and lower the fitness of the infected copepod. The effects on the population level are more difficult to assess. A few studies on the topic have documented that some of these parasites have the capability to control copepod populations in line with the more traditionally accepted mortality factors (predation and starvation). In general, however, our understanding of the role of marine copepods is limited and the exploration of this theme is a research area the just begun to emerge.

Contributed Paper, Thursday 9:45 **161 STU**

Comparision of expression strategy of two brevidensoviruses, *Penaeus stylirostris* densovirus and *Aedes albopictus* densovirus

Hanh T. Pham[(1)], Françoise-Xavière Jousset[(2)], Hiroko Shike[(3)], Jozsef Szelei[(1)], Hanh T. Van [(4)], Jane C. Burns[(3)], Max Bergoin[(2)] and Peter Tijssen[(1)*]
[(1)] INRS-Institut Armand-Frappier, Laval, Québec, H7V 1B7, Canada; [(2)] Laboratoire de Pathologie Comparée, Université Montpellier2, 34095 France; [(3)] Department of Pediatrics, University of California at San Diego School of Medicine, La Jolla 92093-0830; [(4)]Institute of Tropical Biology, Ho Chi Minh City, Vietnam
Address for correspondence: peter.tijssen@iaf.inrs.ca

Based on similar size, organization of coding sequences, and structure of 5'- and 3'-termini of their genome, the *Penaeus stylirostris* densovirus (*Pst*DNV) from shrimps and the *Aedes albopictus* (*Aal*DNV), densovirus from mosquitoes are tentatively classified into the *Brevidensovirus* genus of the *Densovirinae* subfamily (Tijssen et al., in press). Our project encompasses the determination of the X-ray structure of these viruses (completed), obtaining full-length clones, elucidating their expression strategy and devising antiviral control strategies. In the present study, we identified and compared the transcription strategy of PstDNV and AalDNV. We report here a revised transcription map of PstDNV showing that only three mRNAs co-terminating downstream of the rightmost polyadenylation signal (3'end) are produced: two encoding non-structural proteins (left and mid ORFs) and one encoding capsid proteins (right ORF). A similar transcription profile was found for AalDNV but despite these resemblances the two brevidensoviruses differed in their transcription strategies: (i) PstNDV utilized a splicing mechanism resulting in an N-terminal extension to express NS1 protein; (ii) AalDNV used closely overlapping promoters P7 and P7.4 to express NS1 and NS2 proteins respectively, whereas expression of PstDNV NS proteins were under control of two widely spaced promoters (P2 and P12) To complement the results from transcription maps, the activity of their promoters was measured using luciferase assays. The viral promoters were functional in both insect and human cell lines. Interestingly, PstDNV promoters were found to be stronger in HeLa cells than those of AalDNV or the SV40 promoter.

Special Presentation Thursday, 10:30-10:45

Special Presentation, Thursday 10:30 **162**

Pioneer Women in Invertebrate Pathology and their Influence on the Field

Elizabeth W. Davidson
School of Life Sciences, PO Box 874501, Arizona State University, Tempe, AZ 85287-4501, USA
Address for correspondance: e.davidson@asu.edu

Invertebrate pathology has welcomed women into the field from its earliest days, beginning with many women who have been important in the Society since its founding, including four who have served as President, eight as Secretary, four as Treasurer, 9 as Trustee, and many more who have served in other capacities. We will remind ourselves of some of these pioneering women, who courageously entered a field with few female colleagues and made important discoveries in invertebrate pathology.

Contributed Papers
Thursday, 13:30-15:15

Microbial Control 3

Contributed Paper, Thursday 13:30 **163**

Effects of additives and UV protectants on *Plutella xylostella* granulovirus efficacy to control of Diamondback Moth (*Plutella xylostella* Linnaeus).

[1]A. Dezianian, [2]A.S. Sajap, [3]L.W. Hong, [3]D. Omar, [4]H.A. Kadir, [2]R. Mohamed and [4]M.R.M. Yusoh
[1]Department of Plant Protection, Shahrood (Semnan) Agricultural Research Center, Shahrood, Iran. [2]Department of Forest Management, Faculty of Forestry, University Putra Malaysia, 43400 UPM Serdang, Selangor, D, E., Malaysia. [3]Department of Plant Protection, Faculty of Agriculture, University Putra Malaysia, 43400 UPM Serdang, Selangor, D, E., Malaysia. [3]Malaysia Agricultural Research and Development Institute, Serdang, Selangor, D, E., Malaysia
Address for correspondence: dezianian@yahoo.com

The diamondback moth (DBM) *Plutella xylostella*, is known as the most serious pest of crucifers in the world. Regular and repeated use of chemical insecticides for controlling the pest over the years has resulted in DBM developing resistance to all classes of chemical insecticides. *P. xylostella* granulovirus (*Px*GV) was effective for suppressing of the DBM larvae. Susceptibility range of *P. xylostella* granulovirus to ultra violet radiation was evaluated. The results indicate that *P. xylostella* granulovirus was susceptible to ultra violet radiation and lost its virulence after 7 hours of exposure to UV-B radiation under laboratory condition. Virulence of UV treated *P. xylostella* granulovirus were significantly reduced when compared to non-treated *Px*GV as much as 19.64%, 41.53%, 63.17%, 70 and 89% after 5, 15, 30, 60 and 120 minutes exposure to UV radiation, respectively. Adjuvants consisting of Tinopal, molasses, lignin and skimmed milk added separately to *Px*GV suspension significantly improved the residual activity of *Px*GV after exposure to UV radiation. *Px*GV + Tinopal, *Px*GV + molasses, *Px*GV + lignin and *Px*GV + skimmed milk increased 67.78, 65.31, 59.55 and 31.35 % residual activity after being exposed to UV radiation, respectively. The molasses and Tinopal at different virus concentrations before exposure to UV light, significantly increased the residual activity. Molasses showed greatest effects on the larval mortality at all virus concentrations compared to those of Tinopal and lignin before exposure to UV light. The LC_{50} calculated for virus + molasses (5.2×10^4 OBs/ml) before exposure to UV light was 9.2 and 1.75 times lower than lignin and Tinopal, respectively. The results showed that adjuvants used in virus suspension increased viral residual activity and protect virus particles against UV radiation.

Contributed Paper, Thursday 13:45 **164**

The inactivation of *H. armigera* nucleopolyhedrovirus (*Hear*NPV) by chickpea (*Cicer arietinum*) and other legume leaf surface compounds.

David Grzywacz[1], Philip C. Stevenson[2], Reju F. D'Cunha[1] & Aliyu Aminu[1]
[1] Natural Resources Institute, University of Greenwich, Chatham, Kent, ME4 4TB, UK.; [2]Jodrell Laboratory, Royal Botanic Gardens, Kew, Surrey, TW9 3DS, UK.
Address for corespondance: d.grzywacz@gre.ac.uk

Commercial biopesticides based upon *Helicoverpa armigera* nucleopolyhedrovirus (*Hear*NPV) have been registered in Europe, India, China and Africa. They are increasingly used to provide effective and environmentally benign alternatives to synthetic chemicals for controlling *H.armigera*. Trials on chickpea in India, however, have shown that the efficacy and persistence of *Hear*NPV is reduced. The present study compared the efficacy of *Hear*NPV on chickpea compared to cotton and tomato and showed that even in the absence of sunlight the infectivity of virus is inhibited when the virus occlusion bodies (OBs) are exposed to the leaf surface of chickpea for as little as one hour. The degree of inactivation was greater on chickpea than on cotton and the mode of action differed to that reported in cotton. The inactivation was observed with larvae consuming the OB on chickpea leaves but also occurred when OB were removed after exposure to plants and consumed on artificial diet, indicating that inhibition was both surface related and lasting. The present study is investigating the surface chemistry of chickpea and other legume crops to understand the mechanism of NPV inactivation and evaluating concentration changes of leaf surface compounds in response to NPV application. Results have shown that in particular the isoflavonoid sissotrin increases rapidly after spraying and both sissotrin and its aglycone biochanin A reduce the efficacy of the *Hear*NPV OBs. We are also studying similar effects on other legumes such as cowpea (Vigna unguiculata) and pigeonpea (*Cajunus cajunus*).

Contributed Paper, Thursday 14:00 **165**

Control of Oriental Fruit Moth and Codling Moth with a new granulovirus isolate

Markus Züger[1]; Iris Kraaz[1]; Daniel Zingg[1]; Martin Andermatt[1]; Edith Ladurner[2]; Massimo Benuzzi[2]; Marketa Broklova[3]; Gyula Laszlo[4]
[1]Andermatt Biocontrol AG, 6146 Grossdietwil, Switzerland; [2]Intrachem Bio Italia S.p.A., 47023 Cesena, Italy; [3]Biocont Laboratory Ltd., 627 00 Brno-Slatina, Czech Republic; [4]Biocont Magyarorszag Kft., 1139 Budapest, Hungary
Address for correspondence: zueger@biocontrol.ch

In addition to its key role as a pest on stone fruit, oriental fruit moth (*Grapholita molesta*) frequently migrates to pome fruit orchards after the harvest of its original host. Severe damage in apple and pear orchards before harvest is an increasing problem for growers in the Mediterranean Basin. In response to this situation, the Swiss company Andermatt Biocontrol has selected a new granulovirus isolate (ABC-V22) for the combined control of codling moth (*Cydia pomonella*) and oriental fruit moth. With a lethal concentration 50% (LC50) of $1.1x10^3$ OB/g diet in laboratory bioassays on codling moth larvae, ABC-V22 performed equally to the well known and highly effective *Cydia pomonella* Granulovirus (*Cp*GV). Furthermore, the activity of this new isolate reaching a LC50 of $2.9x10^3$ OB/g diet in laboratory bioassays on oriental fruit moth, is a pathbreaking step for the successful control of oriental fruit moth with a new baculovirus product. The promising laboratory results have been confirmed in field trials in Italy, Slovakia and Switzerland in 2010. The effect of the new isolate against oriental fruit moth on stone fruit was a reduction in fruit damage of 68% (on total damage in nectarine, Italy) and 77%

(on active damage in peach, Slovakia) and 90% (on active damage) for codling moth in apple in Switzerland.

Contributed Paper, Thursday 14:15 **166**

Purification of an active fragment of Cry1Ie toxin from *Bacillus thuringiensis*

Shuyuan Guo[1]; Chunlu Zhang[1]; Xiaoyin Lin[1]; Yanrui Zhang [2]; Kanglai He[2]; Fuping Song[2]; Jie Zhang [2]
[1]School of Life Science, Beijing Institute of Technology, Beijing 100081, China, [2]State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100094, China.
Adress for correspondence: guosy@bit.edu.cn; jzhang@ippcaas.cn

The *cry1I* genes from *Bacillus thuringiensis* are a class of special genes with unique characteristics: they are silent in *Bacillus thuringiensis* strains but can be over-expressed in *Escherichia coli*, resulting in a Cry1I-type protein with a molecular mass of approximately 81 kDa. Cry1I- type protein is toxic to Lepidoptera larvae. A truncated Cry1Ie protein, IE648, which corresponds to the first 648 amino acids from the N-terminus of Cry1Ie, was purified from *Escherichia coli* by means of Ni-NTA affinity isolation, Q-Sepharose Fast Flow chromatography, and Superdex-200 size-exclusion chromatography. It was determined using laboratory bioassays that the purified IE648 protein has good insecticidal activity. Heterologous competitive binding assays show that IE648 does not compete with Cry1Ac for binding to the brush border membrane vesicles of the Asian corn borer, and does not compete with Cry1Ac at concentration below 500-fold excess of unlabeled Cry1Ac for binding to the peritrophic matrix of the insect. This result implies that IE648 may be a good candidate as part of a multiple-toxins strategy for the potential control of resistance in insect pests. The method of purification reported here is valuable for further research on the structure and function of IE648 and in evaluating the biosafety of this protein within transgenic plants.

Contributed Paper, Thursday 14:30 **167**

Spore-free antibacterial *Bacillus thuringiensis* delta-endotoxins formulations in integrated pest management

Lidia A. Ivanova[1], Ludmila K. Kamenek[1], Olga U. Shrol[1], Maxim A. Terpilovsky[1]
[1]Ulyanovsk State University, Ecological Department, 42 Lev Tolstoy Street, Ulyanovsk, 432970, Russia
Address for correspondence: maxim@terpilovsky.ru

Chemical and biological insecticides impact on beneficial Hymenoptera insects (Aculeata, Parasitica suborders) in controlling phytophages was studied. Experiments with different insecticides against Colorado beetle and forest pests were conducted over 3 years. The formulations used were Lepidocide (bioinsecticide); Delta-1, Delta-2 (spore free formulations based on cleaved *Bacillus thuringiensis* (Bt) delta-endotoxins); Alpha Cipi, Fury, and Decis (chemical insecticides). Field trials were performed twice on field plots with an area of 5–15 hectares (agricultural fields, mixed forests). Delta-1 (10% W.P., rate of application 0.01 kg/ha) showed equal or higher biological efficacy (92%) than Lepidocide (1 kg/ha) or Decis (0.05–0.06 kg/ha) against nun moths. Delta-2 biological efficacy in controlling Colorado beetle was within 87.2–91.7%. Thus, the results were similar to those for chemical pesticides: Alpha Cipi (87.6%; 0.1 kg/ha), Fury (87.9%; 0.15 kg/ha). Spore free formulations of Delta series proved to be safe to beneficial insects. Species from Scolioidea, Pompiloidea, and Sphecoidea superfamilies disappeared in ecosystems treated with chemical insecticides. Species abundance of Apoidea, Vespoidea, and Ichneumonoidea superfamilies was estimated at 18–28% of control level up to the end of study period. On average, the number of hymenopteran species decreased by 84%. The overall species composition was significantly depleted in comparison with control variant after treatment with chemical pesticides; furthermore, this pattern remained unchanged during the field season. The specimen number and species composition stayed within normal range up to the end of field season after the treatment with Delta series formulations.

Contributed Paper, Thursday 14:45 **168**

Spore-free insecticidal formulations based on cleaved *Bacillus thuringiensis* delta-endotoxins

Ludmila K. Kamenek[1], Dmitri V. Kamenek[1], Vladimir V. Gouli[2]
[1]Ulyanovsk State University, Ecological Department, 42 Lev Tolstoy Street, Ulyanovsk, 432970, Russia, [2]University of Vermont, Entomology Research Laboratory, Bioresearch Center, 661 Spear Street, South Burlington, VT 05405-0105 USA
Address for correspondence: kameneklk@mail.ru

A method for providing and utilizing stable insecticidal spore free formulations based on cleaved *Bacillus thuringiensis* (Bt) delta-endotoxins was developed. The effect of Bt formulations was studied (evaluated) under field conditions in different climatic zones of Russia over 15 years. Formulations showed high levels of efficacy (80–95%) against insect pests of Lepidoptera, Coleoptera, and Hymenoptera orders. The optimal rate of application depends on the method of treatment and varies within 10–150 grams per hectare. Bt formulations are safe for non-target hymenopteran pollination insects and parasites. The method for preparing spore free Bt formulations was awarded the gold medal of the International Salon of Innovations and Inventions (Geneva, Switzerland) in 2005. Detailed results obtained from thorough studies provide an insight into Bt delta-endotoxins mode of action, spectrum of insecticidal activity, and safety to mammals. The effect of such formulations on basic metabolic processes and intestinal microflore condition of mammals was assessed. The ecological risks were evaluated as well.

Contributed Paper, Thursday 15:00 **169**

***Tenebrio molitor* cadherin fragment, a potential additive in *Bacillus thuringiensis* Cry3Aa against vegetable Coleopteran larvae**

Yulin Gao[1]; Zhongren Lei[1]; Juan Luis Jurat-Fuentes[2]; Jeffrey A. Fabrick[3]; Brenda Oppert[4]
[1] State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193, P.R. China, [2] Department of Entomology and Plant Pathology, University of Tennessee, Knoxville, TN 37996, USA, [3] USDA ARS U.S. Arid Land Agricultural Research Center, 21881 N. Cardon Lane, Maricopa, AZ 85138, USA, [4] USDA ARS Center for Grain and Animal Health Research, 1515 College Avenue, Manhattan, KS 66502, USA.
Address for correspondence: zrlei@ippcaas.cn

Bacillus thuringiensis (Bt) is a bacterium that produces toxins used in the control of insect pests. Bt toxins generally are not effective against many beetle pests, limiting the use of Bt toxins in integrated pest management. We evaluated a novel peptide from a toxin receptor in the beetle *Tenebrio molitor* to determine the potential to enhance the activity of Bt toxins against the beetles *Crioceris quatuordecimpunctata*, *Phaedon brassicae* and *Colaphellus bowringi*, serious pests of vegetables in China. The activity of Bt toxin Cry3Aa was increased as much as 15.3-fold in these pests when the peptide was added, compared to the activity of Cry3Aa alone. The data demonstrate that the peptide has potential as an additive in Bt sprays or incorporated into transgenic Bt crops to protect against beetle pests.

Viruses Division Special Symposioum Thursday, 13:30–15:30

Viruses of Forest Insect Pests In Honour of Basil Arif

Organizers: Peter Krell and Kelli Hoover

Symposium Paper, Thursday 13:30 **170**

Viruses of forest insect pests

Peter J. Krell
Department of Molecular and Cellular Biology, University of Guelph, Guelph ON N1G 2W1 Canada
Address for correspondence: pkrell@uoguelph.ca

Many viruses control agricultural insect pests such as cotton bollworm, corn earworm, beet armyworm, and bertha armyworm. Less appreciated are viruses against insect pests of forests and fruit trees and their development as biological control agents. A rather serendipitous example of viruses in biocontrol of a forest insect pest, is that against the European spruce sawfly/Tenthrède européenne de l'épinette (*Gilpinia hercyniae*) introduced to North America in the 1930s. Because of the huge economic impact (loss of 40 million M^3 of spruce timber in Gaspé alone) parasites were imported from Scandinavia to control the outbreak. However, it was a "polyhedrosis" disease, introduced with the parasites, that collapsed the outbreak from 12,000 square miles in 1938 to only a few small pockets by 1945. This "natural" biocontrol demonstrated that viruses too could be developed and used for forest insect pest control. Canada is host to several forest insect pests including Red headed and European pine sawflies Gypsy moth and Douglas fir tussock moth which negatively impact forests and the annual $40 billion forestry industry. The spruce budworm *Choristoneura fumiferana* alone was responsible for the loss of about 70 million ha (out of the 400 million ha total forest in Canada), 10 times more than that lost due to forest fires and harvesting. Several viruses have been developed and commercialized in both the USA and Canada to help control such forest insects, and the research that underpins these advances, some of it from Canada's Basil Arif, will be the focus of this presentation.

Symposium Paper, Thursday 14:00 **171**

Baculoviruses of forest pests – their application from an European perspective

Johannes A. Jehle
Institute for Biological Control, Julius Kühn-Institut, Federal Research Centre for Cultivated Plants, Heinrichtstr. 243, 64287 Darmstadt

The biological control of forests pest insects using highly specific baculoviruses is a promising chance and a technical challenge. In North America, several important forest pest insects are being controlled or have the potential to be controlled using baculoviruses. These include the control of gypsy moth by using *Lymantria dispar* nucleopolyhedroviruses (LdMNPV) [*Alphabaculovirus*], saw flies by using *Neodiprion sp.* nucleopolyhedroviruses (NeleNPV, NeseNPV) [*Deltabaculovirus*], and the spruce budworm by applying *Choristoneura fumiferana* nucleopolyhedrovirus (ChfuNPV) [*Alphabaculovirus*] and *C. fumiferana* granuloviruses (ChfuGV) [*Betabaculoviruses*] and many others. Some of these viruses are registered and commercialized. In Europe, the interest in research and application of baculoviruses in forests declined during the last two decades, presently there is no commercial product available. The presentation will review the developments and future needs in baculovirus research to become more attractive for biocontrol of forest pests.

Symposium Paper, Thursday 14:30 **172**

Lymantria dispar nucleopolyhedrovirus as a microbial control agent of the forest insect pest *L. dispar*

Kelli Hoover[1] and James M. Slavicek[2]
[1]Department of Entomology, Penn State University, 501 ASI Building, University Park, PA 16802 USA
[2]USDA Forest Service, 359 Main Road, Delaware, OH 43015 USA
Address for correspondence: kxh25@psu.edu

The gypsy moth, *Lymantria dispar*, was introduced into the United States in 1869 and currently is present in 19 Northeastern and Midwestern states. The *Lymantria dispar* nucleopolyhedrovirus (LdMNPV) was developed and registered as a microbial control agent for the gypsy moth by the US Forest Service and is currently used primarily in areas in the United States where there is concern about non-target effects of Bt on endangered Lepidoptera. The LdMNPV is a type II Alphabaculovirus, and of the sequenced lepidopteran NPVs to date is among those with the lowest AT content and the largest genomes. This virus contains the 62 common genes found in the lepidopteran-specific NPVs and GVs. Our recent studies have focused on the function of two LdMNPV viral enhancing factor proteins during initial stages of infection, and the results indicate that there is a function other than degradation of peritrophic matrix proteins. We will provide an overview of LdMNPV use to control the gypsy moth in the United States, early events during viral infection of larvae, including anti-viral defenses of the host, and our recent studies on the viral enhancin genes.

Symposium Paper, Thursday 15:00 **173**

Baculoviruses and the population cycles of two insect herbivores of balsam fir

Christopher J. Lucarotti
Natural Resources Canada, Canadian Forest Service - Atlantic Forestry Centre, 1350 Regent Street, Fredericton, New Brunswick, Canada, E3C 2G6.
Address for correspondence: clucarot@nrcan.gc.ca

The balsam fir sawfly (*Neodiprion abietis*) and the spruce budworm (*Choristoneura fumiferana*) are two insects that feed on balsam fir (*Abies balsamea*) and experience population outbreak periodicities of approximately 15 and 35 years, respectively. Balsam fir sawfly population outbreaks can cause defoliation over tens of thousands of hectares, most recently (since 1990) in precommercially thinned stands of balsam fir forests in Newfoundland and Labrador. During the last spruce budworm outbreak (1978–1992), some 58 million hectares of boreal forest were adversely affected, mostly in eastern Canada. Balsam fir sawfly populations are regulated, almost exclusively, by a gammabaculovirus (NeabNPV), but spruce budworm populations are impacted by a large and diverse array of pathogens and parasites where an alphabaculovirus (CfMNPV) and a betabaculovirus (ChfuGV) appear to play only minor roles. Balsam fir sawfly larvae feed openly and in groups only on balsam fir foliage that is 1-year-old and older thus facilitating the spread of the contagious, midgut-infecting NeabNPV. Budworm larvae, however, overwinter as second instars in hibernacula, mine into needles in spring, and individually construct feeding tunnels in the expanding buds as third instars. This cryptic and solitary habit likely limits opportunities to transfer CfMNPV and ChfuGV horizontally. Spruce budworms may also feed on white (*Picea glauca*) and black spruce (*Picea mariana*) in addition to balsam fir. The differing habits and evolutionary histories of the balsam fir sawfly and spruce budworm have likely influenced not only the roles of the baculoviruses affecting them but also their susceptibility to other pathogens and parasites.

Symposium (Bacteria) Thursday, 16:00–18:00

Resistance to Bt Crops

Organizers: Juan Ferre and Juan Luis Jurat-Fuentes

Symposium Paper, Thursday 16:00 **174**

Field-evolved resistance to Bt crops

Bruce E. Tabashnik[1], Xianchun Li[1], and Yidong Wu[2]
[1]Department of Entomology, University of Arizona, Tucson, Arizona 85750, USA
[2]College of Plant Protection, Nanjing Agricultural University, Nanjing 210095, China
Address for correspondence: brucet@cals.arizona.edu

Evolution of insect resistance threatens the continued success of transgenic crops that produce *Bacillus thuringiensis* (Bt) toxins to kill some key pests. Field-evolved resistance entails a genetically based decrease in susceptibility of a population to a toxin caused by exposure of the population to the toxin in the field. Although many pest populations remain susceptible, field-evolved resistance has been reported in some populations of at least six major pests. For Bt corn, resistance has been reported for *Spodoptera frugiperda* to Cry1F in Puerto Rico, *Busseola fusca* to Cry1Ab in South Africa, and *Diabrotica virgifera virgifera* to Cry3Bb in the midwestern US. For Bt cotton, reported cases of resistance include *Helicoverpa zea* to Cry1Ac and Cry2Ab in the southeastern US, *Pectinophora gossypiella* to Cry1Ac in India, and *Helicoverpa armigera* to Cry1Ac in China. The cumulative number of pest species with field-evolved resistance increased from zero for the first 5 years (1996-2000), to one for the first 10 years (1996-2005), and to six for the first 15 years (1996-2010). Consistent with theory, field data indicate that abundant refuges of non-Bt host plants can delay resistance, particularly when resistance is inherited as a recessive trait. To thwart pest resistance, some transgenic crops produce "pyramids" of different Bt toxins targeting the same pest, including Bt vegetative insecticidal proteins (Vips). Future transgenic crops may use RNA interference and modified Bt toxins engineered to kill pests resistant to native Bt toxins. Knowledge of insect resistance to Bt crops can help to minimize risks and enhance benefits.

Symposium Paper, Thursday 16:30 **175**

Adaptive management of resistance to Bt-cotton in Australian *Helicoverpa* spp.

Sharon J. Downes[1]; Rod Mahon[2]; Tracey Parker[1]; Bill James[2]
[1]CSIRO Ecosystem Sciences, Australian Cotton Research Institute, Locked Bag 59, Narrabri, NSW 2390 Australia, [2]CSIRO Ecosystem Sciences, Black Mountain Laboratories, PO Box 1700, Canberra, ACT 2601 Australia
Address for correspondence: Sharon.Downes@csiro.au

In Australia, monitoring *Helicoverpa* species for resistance to the Cry2Ab toxin in second generation *Bacillus thuringiensis* (Bt) cotton has fulfilled its intended function: to warn of increases in resistance frequencies that may lead to field failures. Prior to the widespread adoption of two-gene Bt cotton (Bollgard II), the frequency of Cry2Ab resistance alleles was at least 0.001 in *H. armigera* and *H. punctigera*. In the six years hence, there has been a statistically significant increase in the frequency of alleles conferring Cry2Ab resistance in field populations of *H. punctigera*. In this presentation we review the history of deploying and managing resistance to Bt cotton in Australia, outline the characteristics of the isolated resistance that likely impact on resistance evolution, and use a simple model to predict likely imminent resistance frequencies. We then discuss potential strategies to mitigate further increases in resistance frequencies, including the release of a third generation product that utilizes the novel vegetative insecticidal protein Vip3A. The robustness of the Vip3A inclusive variety will depend on resistance frequencies to Vip3A and to Cry2Ab when it is released (anticipated 2014) and the efficacy of Vip3A throughout the season. The area planted to Bt-crops is anticipated to continue to rise worldwide and many biotechnical companies intend to add Vip3A to existing products; therefore the strategies being considered in Australia are likely to relate to other situations.

Symposium Paper, Thursday 17:00 **176**

Resistance to Bt maize in *Spodoptera frugiperda*: Lessons from Puerto Rico

Nicholas P. Storer, Ed King, Gary D. Thompson
Dow AgroSciences LLC, 9330 Zionsville Road, Indianapolis, IN 46268, U.S.A.
Address for correspondence: nstorer@dow.com

Transgenic maize (*Zea mays*) event TC1507 produces the Cry1F protein from *Bacillus thuringiensis* Berliner (*Bt*) *var. aizawai* to provide protection from feeding by several important lepidopteran pests, including *Spodoptera frugiperda* (J. E. Smith). High-level resistance to Cry1F in this species was recently documented in Puerto Rico. Multiple factors likely contributed to this resistance event, including aspects of the tropical island biogeography, maize crop management practices by farmers, pest biology, and variation in pest sensitivity to Cry1F. The resistance is highly recessive, likely controlled by a single allele, and does not confer significant cross-resistance to other *Bt* proteins used in transgenic maize lines. The factors that contributed to this incident are unique to North America and on-going monitoring programs indicate that the mainland US populations are still fully sensitive. Certain parallels are anticipated in other tropical geographies however, where maize is intensively cultivated but insect dispersal is limited. Proactive resistance management (including the use of trait pyramids combining Cry1F with other *Bt* proteins, and the planting of structured refuges where appropriate) will be important in ensuring prolonged durability of this important pest management tool.

Symposium Paper, Thursday 17:30 **177**

Field failure of first-generation Bt Cotton documented with pink bollworm in Gujarat State, India

William Moar[1]; Graham P. Head[1]; John Greenplate[1]; K.S. Mohan[2]; K.C. Ravi[2]
[1]Monsanto Company, 800 North Lindbergh, Creve Coeur, MO 63167, [2]Monsanto Research Centre, 44/2A Vasanth's Business Park, Bellary Road, NH – 7, Hebbal, Bangalore 560092, India.
Address for correspondence: william.moar@monsanto.com

Unusual survival events of *Pectinophora gossypiella* (Saunders) (pink bollworm, PBW) in Bollgard hybrids were documented during the 2009 growing season in the Indian state of Gujarat. An unusual survival event was one in which inspected bolls were infested by live PBW larvae at the rate of 10% or greater. In Gujarat in 2009, Bollgard fields were sampled for PBW damage/infestation. A large proportion of sampled fields met the criterion for unusual survival with numerous bolls containing surviving larvae which had reached at least the 4th larval instar. Subsequent populations of PBW derived from these field-collected larvae survived at high levels in in-vitro bioassays against diagnostic doses of Cry1Ac (1µg/ml; 10µg/ml). These populations were considered resistant to Cry1Ac. In similar assays, these same Cry1Ac-resistant field populations were found to be fully susceptible to Cry2Ab (no survivors at the diagnostic concentration of 10µg/ml). Cry2Ab is the second Cry protein expressed in second-generation Bt cotton (Bollgard II). Factors possibly contributing to the development of Cry1Ac-resistant populations in the field in Gujarat include the illegal deployment of unapproved hybrid Bt events with lower Cry1Ac levels and the accompanying failure to plant refuges adequate to produce sufficient susceptible PBW moths.

Microsporidia 2

Contributed Paper, Thursday 16:00 **178**

Persistence, stability and co-occurrence of *Knellallenhazia solenopsae, S. invicta* virus 1 (SINV-1), and SINV-2 infections across years in Louisiana

Maynard L. Milks; James R. Fuxa; Arthur R. Richter.
Department of Entomology, Louisiana State University, Baton Rouge, LA, USA 70803
Address for correspondence: mmilks1@lsu.edu

Red imported fire ants *Solenopsis invicta* (Buren) were accidentally introduced into the US from South America in the 1930's and have spread throughout the southern states becoming serious agricultural and urban pests. Until now, *S. invicta* has predominantly been controlled with chemical insecticides. Unfortunately, these compounds often only provide temporary control, are not economical for use over large areas, and may have harmful side-effects for the environment. Several microbial pathogens including the microspordium *Knellalenhazia solenopsae* and 3 RNA viruses, *S. invicta* virus 1 (SINV-1), SINV-2, and SINV-3 have been recovered from *S. invicta* raising the possibility that one, or a combination of them, may be used as biological control agents. Insect pathogens, like other organisms, occupy specific ecological niches and a thorough understanding of their epizootiology – distribution and abundance; spread; and persistence – is a prerequisite to their successful use as bioinsecticides. In 2003, we surveyed 165 *S. invicta* populations to determine the prevalence and distribution of *K. solenopsae* in Louisiana. The current study is a follow up and consists of three sections. First, we used archived samples from the 2003 survey to examine the distribution of SINV-1 and SINV-2 across Louisiana. Second, in 2007, we re-surveyed 57 sites to ascertain the stability, persistence and co-occurrence of the three pathogens over a broad geographic scale. Third, we sequenced the internal transcribed spacer region of *K. solenopsae* collected in different parts of the state and in different years to gauge the extent of genetic variability in the microsporidium.

Contributed Paper, Thursday 16:15 **179**

Effects of a microsporidium from the convergent lady beetle, *Hippodamia convergens* Guérin-Méneville, on the endoparasitoid *Dinocampus coccinellae* (Schrank)

T. Saito[1] and S. Bjornson[2]
[1]Vineland Research and Innovation Centre, 4890 Victoria Ave. N., Box 4000, Vineland Station, ON CANADA L0R 2E0;
[2]Department of Biology, Saint Mary's University, 923 Robie Street, Halifax, NS, CANADA B3H 3C3

Convergent lady beetles, *Hippodamia convergens* Guérin-Méneville are host to the braconid endoparasitoid, *Dinocampus coccinellae* (Schrank) and at least one species of microsporidia. The objective of this study was to examine the effects of the microsporidian pathogen *Tubulinosema hippodamiae* on wasp development (30-day trials) and host choice. Uninfected *D. coccinellae* were provided uninfected and *T. hippodamiae*-infected host beetles under laboratory conditions. All wasp progeny that developed within (and emerged from) *T. hippodamiae*-infected beetles were infected with the microsporidium (100% transmission; n =46). These infected wasps were also provided uninfected and infected beetles as hosts. The duration of endoparasitoid development (from egg deposition in the host until adult eclosion) was recorded for uninfected and infected wasps in both uninfected and infected beetles. Mean development did not differ significantly among wasps from any of the four treatments. Beetles were dissected at the end of the 30-day trial if *D. coccinellae* wasps failed to emerge of if the host died prior to the end of the trial. A significantly greater proportion of beetles stung by microsporidia-infected wasps did not contain an endoparasitoid larva (χ = 7.35, df = 1, P = 0.007) when compared to those stung by uninfected wasps, suggesting that the pathogen affects the viability of the endoparasitoid or its eggs. *T. hippodamiae* was transmitted from infected wasps to their progeny (second consecutive generation) when the former were provided with uninfected beetles as hosts (n=12). Examination of paraffin-embedded *D. coccinellae* adult tissues revealed an extensive microsporidian infection. Uninfected and infected wasps were provided a choice of uninfected and infected beetles during a 30-minute host preference trial (choice test). *D. coccinellae* adults pursued, took an ovipositional stance, and attacked uninfected beetles more often than they did microsporidia-infected hosts; however, these observations did not differ significantly (P >0.05).

Contributed Paper, Thursday 16:30 **180**

Characterization of nosemosis in the sugarcane borer, *Diatraea saccharalis*, and its parasitoid *Cotesia flavipes*

Renata A. Simões, Júlia R. Feliciano, Letícia G. Reis, Italo Delalibera Jr.
Universidade de São Paulo (ESALQ/USP), Av. Pádua Dias 11, C.P. 9, Piracicaba/SP, Brazil, CEP 13418-900,
Address for correspondence: italo@esalq.usp.br

Diatraea saccharalis (Lepidoptera: Crambidae) is the major pest of sugarcane in Brazil. This pest is controlled by inundative releases of the larval parasitoid *Cotesia flavipes* (Hymenoptera: Braconidae) in more than 3 million hectares of sugarcane every year. However, in the commercial parasitoid rearing system, infection of *D. saccharalis* by *Nosema* sp. is the biggest concern. In this study, we characterized the impact of *Nosema* sp. in the biological parameters of *D. saccharalis* and *C. flavipes*. Morphological and Molecular characterization were carried out by TEM and partial 18S and 28S rRNA sequence analysis, respectively. The estimated LD_{50} were 5.6 and 1230 spores for 1st and 3rd caterpillar instars, respectively. The LT_{50} ranged from 42 to 9 days when treated with concentration from 10^1 to 10^7 spores/1st instar caterpillar. For the 3rd instar, LT_{50} ranged from 49 to 32 days at concentrations from 10^2 to 10^7 spores/caterpillar. Parasitoids originated from infected caterpillars presented longer larval and pupal developmental times, lower larval and pupal survival, lower adult emergence and smaller body size, and this parameters were correlated with the host infection level. The pathogen is vertically transmitted throughout the parasitoid life cycle. Host range study was carried out with six Noctuid species and revealed that *Nosema* sp. was pathogenic to *Anticarsia gemmatalis*, *Pseudoplusia includens* and *Spodoptera albula* causing higher infection levels in the first two species. *Heliothis virescenses*, *S. cosmioides* and *S. frugiperda* were not susceptible to the pathogen. *Nosema* sp. is a virulent pathogen against 1st instar *D. saccharalis* caterpillar and can also negatively affect *C. flavipes* in mass rearing facilities.

Contributed Paper, Thursday 16:45 **181**

Temperature affects development of the microsporidium *Nosema lymantriae* and disease progress in the host *Lymantria dispar*

Dörte Goertz, Sieglinde Pollan, Gernot Hoch
BOKU University of Natural Resources and Life Sciences, Vienna; Department of Forest- and Soil Sciences, Hasenauerstr. 38, 1190 Vienna, Austria
Address for correspondence: doerte.goertz@boku.ac.at

Nosema lymantriae Weiser, an important microsporidian pathogen of *Lymantria dispar* L., infects most tissues of host larvae and uses two main horizontal transmission pathways. Spores are released either with feces from living larvae or from decomposing cadavers. The onset of spore release influences horizontal transmission of *N. lymantriae*. This study describes effects of temperature (18°C,

21°C, 24°C) on development of *N. lymantriae* and progress of infection in *L. dispar*. Vegetative stages and primary spores of *N. lymantriae* appeared 4 days earlier in infected larvae reared at higher temperatures. Environmental spores were found in the Malpighian tubules 12 days post infection (dpi) at 21°C and 24°C, and 16 dpi at 18°C, respectively. Infected larvae reared at 24°C began to release spores with feces 7 days earlier than larvae reared at 18°C. The total amount of spores released with feces within the first six days during the 5th instar was significantly higher at higher temperature. Infected larvae reared at 24°C died about two weeks earlier than larvae reared at 21°C or 18°C; microsporidiosis caused 100% larval mortality at all temperatures. When male larvae were reared at 21°C, the fresh mass of their cadavers and the total amount of spores produced was significantly higher than for larvae reared at 18°C or 24°C. The effect of temperature was not significant for female larvae. The total amount of spores produced in a larva varied between $1.0x10^{10}$ and $1.5x10^{10}$ spores per female and between $5.9x10^{9}$ and $1.4x10^{10}$ spores per male cadaver. We will use these results to model and estimate the effects of temperature on horizontal transmission success of *N. lymantriae*.

Contributed Paper, Thursday 17:00 **182**

Evaluating methods of disinfecting *Nosema ceranae*-contaminated comb

Abdullah Ibrahim; Andony P. Melathopoulos; Stephen F. Pernal
Beaverlodge Research Farm. Agriculture and Agri-Food Canada, AB. P.O. Box 29, Beaverlodge, Alberta T0H 0C0
Address for correspondence: Abdullah.Ibrahim@AGR.GC.CA

Disinfection of comb contaminated with spores of *Nosema apis* is an effective method for preventing spread of this parasite among honey bee colonies (*Apis mellifera*). We hypothesized that methods used to suppress *N. apis* would also be effective against *Nosema ceranae*, a new and potentially devastating parasite of honey bees. To test this hypothesis, we sprayed *N. ceranae* spores onto brood comb surfaces (4.51×10^{8} spores/ hive) and compared three different disinfection methods (*n*=12 colonies/ treatment): electron beam irradiation, acetic acid fumigation and heat (49°C). Two groups of untreated colonies were also used, those with spore-inoculated frames and those not inoculated. Colonies were evaluated between May 2009 and August 2010 for spore infection levels, colony productivity and survival. Fumigation, heat and irradiation suppressed spore production during the spring peak of infection in 2009, shortly after the start of the experiment. The irradiation treatment, however, suppressed spore development to levels similar to that of uninoculated colonies for the duration of the experiment. Two months after establishment, significantly greater populations of adult bees were found on irradiated comb compared with heat-treated comb, with the remaining treatments being intermediate. Little variation was found in the area of sealed brood or adult bee populations among treatments during other periods. Irradiated and acetic acid-fumigated colonies produced the greatest amount of honey. All irradiated colonies survived, while almost half of the heat-treated or inoculated colonies perished before the end of the experiment. Irradiation is the most effective method for disinfecting *N. ceranae*-contaminated comb.