Disease Perspectives from the Global Crustacean Fishery

Plenary Symposium, Monday 10:30

Crustacean diseases – A Canadian perspective
Rick Cawthorn
Department of Pathology and Microbiology, AVCLSC

Plenary Symposium, Monday 11:00

Crustacean diseases – A US perspective
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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

Crustacean Diseases – an EU Perspective
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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 disease 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.

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 (Thomus 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 lobster forms 18% and shrimp 13% (2006-09 exports for Australia worked out 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.
How to approach senior scientists for joint grant writing

Jürgen Eilenberg

Making sure your grant is funded

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A winning proposal: effective grant writing for early career researchers

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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.

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

Monsanto Company, 700 Chesterfield Parkway West, Chesterfield, Missouri 63017

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.

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

Lixin Dou1, 2, Lili Qiu1, Jie Zhang1, Shuliang Feng2, Fuping Song1, 3, Dafang Huang4
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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-transcribed. However β-galactosidase assay demonstrated that the intergenic region between orf1 and cry8Ea1 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

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Student Workshop
How to Write Grant Proposals
Organizers: Sastia Putri and Kelly Bateman

Student Workshop

Student Workshop

A winning proposal: effective grant writing for early career researchers

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

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3Biotechnology Research Institute, CAAS, China
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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-transcribed. However β-galactosidase assay demonstrated that the intergenic region between orf1 and cry8Ea1 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

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between orf1 and cry gene existed a promoter. Beta-galactosidase assay showed that transcriptional activity of Porf1 was abolished in the sigH mutant while transcriptional activity of Porf2 remained significantly decreased in the sigH mutant. Transcriptional start sites directed by Porf1 and Porf2 were determined by 5'-smarter race method respectively. The -35 and -10 regions of Porf1 and Porf2 showed highly sequence similarity with $\sigma^7$ and $\sigma^H$ recognized motifs. These results indicated that Porf1 was controlled by $\sigma^7$ factor and Porf2 was controlled by $\sigma^H$ factor.

Contributed Paper, Monday 14:45

Cyt1A of Bacillus thuringiensis restores Bacillus sphaericus Bin toxicity via large microvillar lesions, not cation channels, in Bin-Resistant larvae of Culex quinquefasciatus
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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 fluorecent 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 $\alpha$-glucosidase receptor. In sensitive larvae of Cx. quinquefasciatus, 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

The 60-kDa protein encoded by orf2 in the cry19A operon of Bacillus thuringiensis subsp. jegathesian functions like a C-terminus of the 135-kDa Class Cry proteins
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The cry19A operon of Bacillus thuringiensis subsp. jegathesian encodes two proteins, Cry19A, a 75-kDa mosiquitocidal 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 acrystatelliferous 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 cry1A promoters combined with the 5’ STAB-SB 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 cryIC 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

Using multi-omics approaches to elucidate the bases of resistance to Bti in Aedes aegypti
Guillaume Tetreau1, Margot Paris1, Krishnareddy Bayyareddy2, Christopher Johnson1, Michael J. Adang2,3, Jean-Philippe David1, Laurence Després1
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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 litter 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  
\textbf{14}  
\textbf{Fruit and Shoot Borer Resistant Transgenic Bt Brinjal in India}  
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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 (ABSP-II) of USAID and Department of Biotechnology, Govt. of India with the involvement of M/s. Maharashtra Hybrid Seeds Company (Maheco) (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 benefit 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, farmers will have option to save seeds to subsequent crop. The transgenic Bt brinjal contains cry 1Ac 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. 

Brinjal is shaping India regulatory system and would make way for transgenic food events in days ahead.

Contributed Paper, Monday 15:45  
\textbf{15}  
\textbf{Field level Resistance in bollworms to Bt cotton in India}  
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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 new clues to identify the receptors of the midgut cells involved. 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, \textit{Junonia coenia DNV} on a susceptible host the Lepidoptera \textit{S. frugiperda}. Comparative crystallographic analysis of \textit{JcDNV} and the closely related \textit{GmDNV} revealed that few residues on the capsid might be involved in host specificity (Bruemmer A. et al., 2005). To test this hypothesis, several \textit{JcDNV} carrying mutated residues were constructed and used to infect \textit{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 \textit{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 the receptors of the midgut cells involved.

Contributed Paper, Monday 14:15  
\textbf{17}  
\textbf{Molecular characterization of \textit{Autographa californica} multiple nucleopolyhedrovirus ORF43 null mutant}  
Xue Ying Tao1, Jae Young Cho1, Yong Wang1, Jae Su Kim2, and Yeon Ho Je1  
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\textit{Orf43 (ac43)} of \textit{Autographa californica} multiple nucleopolyhedrovirus (AcMNPV) is a highly conserved baculovirus gene whose function is unknown. To determine the role of \textit{ac43} in baculovirus life cycle, we used a new AcMNPV bacmid (bAc-MK) and generated \textit{ac43} deletion virus (ac43KO) by using the plasmid capture system (PCS). After transfection into \textit{Spodoptera frugiperda} cells, \textit{ac43KO} produced significantly different OBs which with much larger size; and especially had much single nucleocapsids compared to Ac-MK. Furthermore, \textit{ac43KO} bacmid led to a defect in transcription and expression of polyhedrin, which result in less OBs production. However, \textit{ac43KO} didn’t affect BV production since there’re no remarkable difference of BV titer in both \textit{ac43KO} and Ac-MK. These results demonstrate that \textit{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 1,2, David Thiel mann 1, Martin Erlandson 1,2

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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 Trichoplusia 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 intrahemocellically. 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 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

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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

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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

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

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

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Glycosylation is commonly considered of significant meaning for the functions of glycoprotein during the viral life-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 F1 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 were conducted by insertion of the 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 (rHasSNPVs) 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 seemsly facilitated virus entry into host cells. The mutation as N526 impaired virus production and infectivity as well as fusogenicity 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 22

Micorbial Control 1

Contributed Paper, Monday 14:00 24

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

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The cotton bollworm, Helicoverpa armigera, is a serious global agricultural pest. Its predation on crop plants has been successfully controled by the application of insecticidical 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, Pseudomonas fluorescens or Escherichia coli. We have examined the levels of major components of phenoloxidase cascade, prophenoloxidase (PPO) and prophenoloxidase-activating enzyme (PPE) upon challenge with P. fluorescens/E. coli. The extent of upregulation of PPO/PPE is extremely divergent upon different bacterial challenge. The inter-relationship between PPO-PPE and coordinated modulation has been examined at various levels of microbial density. The interaction of PPO and PPE 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.
Interaction between a beetle and its pathogen: do Asian longhorned beetles behaviorally fever?

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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.

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

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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 autocidal dissemination technique whereby self-contaminated or autoparasitized 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 to 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.

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

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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. Optimum external sterilization technique using sodium hypochlorite on 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 (S$) than sterile soil (SS). Additional treatments of grub immersion in 10% sodium thiosulfate (instead of water) and sodium thiosulfate (instead of water) as external sterilization, as well as effects on development in sterile conditions, are also under investigation.
A push-pull biocontrol strategy involving Chinese cabbage, red clover Entomophthora muscae and the cabbage- and turnip root fly
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The use of Chinese cabbage as a trap crop where insect pathogenic fungi may proliferate 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.

Potential synergism between Beauveria bassiana and plant extract of Ginkgo biloba against the two-spotted spider mite, Tetranychus urticae (Acari: Tetranychidae)
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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 LC50 value of B. bassiana (10 1 conidia/ml) were considered in assays. The mites were sprayed with 1.5 ml of each treatment 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 12,9, 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.

Fungus in an oily residue – the perfect solution for a healthy home…
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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/buts. 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 unfumigated 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.

Deciphering the genetic and biological plasticity of Cydia pomonella granulovirus (CPGV)
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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, -112, -E2, -S, -I07. These analyses showed that the isolates belong to different genome types and differ in single nucleotide 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 bioassays and in the field. We aim to elucidate this complexity in the interaction of different 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

The Agrotis baculovirus complex: multiple viruses for multiple pests
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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 (AgeBV) were isolated from the common cutworm A. segetum. 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 (LC50) 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

The ecology of Spodoptera exigua (Lepidoptera: Noctuidae) in 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. exigua 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

SeMNVP reactivation through stress factors in covertly infected Spodoptera exigua
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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 (SeMNVP) were mated and the subsequent generation (F1) tested for virus reactivation in the second instar. A number of treatments were tested: i) chemical stressors: 1-0.1% copper sulfate, 1% iron sulfate, 1-0.1% hydroxyamine, 2% Tinopal, 1 ppm paraquat dichloride, 1 ppm sodium selenite; ii) inoculation with: SeMNVP-US2, Chrysovertis chalceides NPV (non permissive) and Bacillus thuringiensis (spores and crystals); and iii) rearing temperatures of 18°C and 28°C. 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 descendants of adults that acquired the infections under semi-field conditions.

Contributed Paper, Monday 17:30

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

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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 thought 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 caterpillar, Malacosoma californicum pluviale. Annual collections of larvae from field population sites in British Columbia have been reared in the laboratory to assess the level of covert 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 the moth and the prevalence and level of persistent virus follows the host population density trajectory.

Contributed Paper, Monday 17:45

Horizontal gene transfers between baculoviruses and entomopoxviruses

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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 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

Genome sequence of a nudivirus from the crane fly Tipula oleracea

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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 17:30-18:30

Fungi 1

Contributed Paper, Monday 16:30

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

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Pollen beetles Meligethes aeneus (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 RNA 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.

The progression of disease in ectotherms is strongly influenced by environmental temperature; however, exactly what temperature 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.

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

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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 florisorum. Chelostoma florisorum 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. florisorum* and carried out several experiments to test our hypothesis. We switched the provisions of *C. florisorum* 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 vivo* spore germination of pathogenic *Ascosphaerae*. The evaluation of a narrow host plant diet in bees is therefore 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

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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 *Conidobolus 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 access to the hemolymph and exit for sporulation. Further, the entomophthoralean fungus secretes 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

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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.

A novel nuptially-transmitted fungal symbiont of Tenebrio molitor

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The yellow mealworm, Tenebrio molitor, harbors a symbiont that has spores with a thick, laminated wall and infects the fat body and ventral nerve cord 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

Construction of cDNA library and cloning of V-ATPase subunit B in midgut from Helicoverpa armigera (Hübner)
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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 2x10⁶ 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. Among 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 tags of Vaccuolar ATP enzyme were found. Vaccuolar 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
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Bacillus thuringiensis (Bt) is a rod shaped, gram positive, sporforming 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 BBMV’s. 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
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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 activity 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.
Differential Gene Expression in the Gypsy Moth (Lymantria dispar) Larval Midgut in response to Bacillus thuringiensis (Bt) infection

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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 ~10^6 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 filtering 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.

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

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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 His41 and Gln231 were respectively replaced by Asn41 and Thr231 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 may be 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**

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*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 the all 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**

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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, 453QAAG455 or 443AVYT446 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 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 453QAAG455 library and a mutant from 443AVYT446 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 Ostrinia furnacalis (Guenée)**

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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 borer exhibits high levels of cross-resistance to Cry1Ah as well as Cry1Ac and Cry1F, no cross-resistance to Cry1H has been detected. Understanding the molecular mechanism of cross-resistance in Asian 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 Cry1le binding proteins from the brush border membrane vesicles (BBMV) from Asian corn borer larvae. Bt binding proteins in the BBMV were detected by 2D gels 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-AhR BBMV when compared to ACB-Bt BBMV in ligand blots using biotinated Cry toxins with equal amounts of protein loaded. Interestingly, Cry1le toxin binding to the V-type proton ATPase catalytic subunit A was not detected in ACB-Bt. 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 Cry11Aa toxin to Aedes aegypti.**

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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, η 16.7 nM. Based on these results, we investigated if the cadherin-like receptor is involved in the in vivo toxicity of Cry11Aa toxin to *Ae. aegypti*. First dsRNA of 507 or 781 bp were made that targeted
the Aedes cadherin. Ae aegypti larvae were then fed effectence-encapsulated dsRNA, and at early fourth instar the larvae were then treated with Cry11A at the LC50 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.

We describe surveys conducted to detect Wolbachia in native Agrius 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 Plagionomophora sulcata followed by Atanycolus spp. and the least abundant parasitoids were Balbiella 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 obligate symbiosis, which may result from coevolution 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 infected Wolbachia, discuss patterns of genetic diversity in demographic history and comment on possible selective sweep and its impact on P. sulcata reproduction.

The Cry11Aa protein is more toxic to Aedes aegypti larvae than other proteins produced in Bacillus thuringiensis subs. 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 Kd 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 Cry11Aa 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 comparative 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.

The Vip3Aa16 protein secreted by Bacillus thuringiensis BNS3 during the vegetative stage of growth displays differential toxicity towards Spodoptera frugiperda and S. exigua, with LC50 values of 35 (21 – 57) and 198 (79 – 804) ng/cm², 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 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.
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 mutagenized 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 (e.g. receptor binding). 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
Improving the chitinolytic activity of chitinase genes by combining toxins with crystal genes of Bacillus thuringiensis
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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 degenerate 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 (BUL200) 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 karstaki and israelensis strains of Bacillus thuringiensis. The insecticidal activity of engineered Bt strains were studied with Galleria mellonella, Spodoptera littoralis and Drosophila melanogaster larvae. 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
The nematocidal toxin Cry5B from Bacillus thuringiensis is a pore-forming toxin required for toxic actions
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Cry5B is a 140-kDa protein produced by Bacillus thuringiensis. It is activated in C. elegans nematode gut into a 59-kDa toxin which elicits major morphological and cellular alterations in the host gut resulting in death within 5-6 days (LC50 ~ 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 to bacterial functional defense, including the JNK MAPK pathway and the API 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 ratio Pk/Pcl = 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
Discovery and characterization of a novel Bacillus thuringiensis Cry1B-type insecticidal protein
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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
Variation of host cell components required for toxic actions of different subclasses of Cry1 toxin.
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Cry toxic is the insecticidal protein toxin produced by Bacillus thuringiensis (Bt). However, mechanism of action of the toxin may be more complicated than have been estimated 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 susceptibility 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 susceptible 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 susceptible to 500 nM Cry1La toxins. These indicated these Cry1La, 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 cell which was originally. We will report results of these experiments and discuss on the importance of the ABC transporter class C for Cry1 toxins’ activity.

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 malpighian tubules 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 culmarr 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) which made larvae stop eating was given to the larvae, any swelling culmarr cells were not observed TUNEL highly-positive culmarr cells were detected by the staining at 4 to 24 h post ingestion, resulting in decrease of culmarr cell density in the tissue and increase of cavity space of the goblet cells. Interestingly, 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.

Heliotline 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 B20
Bombbyx mori midgut epithelial cells’ reaction induced by Cry1Aa includes both or either of osmotic swelling and apoptosis depending on toxic condition.
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The cleavage of the loop between alpha-3 and alpha-4 helix is critical for insecticidal activity of Cry8Da
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Antimicrobial activity of bacteriocins from Bacillus thuringiensis subsp. cameroni
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Bacillus thuringiensis is characterized by its ability to synthesize crystal toxins and also to produce bacteriocins such as thuricin, tochicin, entomocin and bacthuricin. The present work,

Poster / Bacteria, Monday 16:00 B23
Antimicrobial activity of bacteriocins from Bacillus thuringiensis subsp. cameroni
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Bacillus thuringiensis is characterized by its ability to synthesize crystal toxins and also to produce bacteriocins such as thuricin, tochicin, entomocin and bacthuricin. The present work,
for the first time, describes the biological activity of bacteriocins from \textit{B. thuringiensis} subsp. \textit{cameroun}. Supernatant which was produced generating broth culture of \textit{B. thuringiensis} subsp. \textit{cameroun} had antimicrobial activity against \textit{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) giving the thermal instability. A group of FPLC fractions had antimicrobial activity against \textit{B. thuringiensis} subsp. \textit{cameroun}, \textit{palmyrenensis}, \textit{israelensis}, 1-3, \textit{morrisoni}, \textit{toguchini} and \textit{karustki}, and \textit{B. cereus} ACTC21768, ATCC14579 and NRRLB-369. Interestingly, when the supernatant was individually incorporated into the liquid cultures of mosquitocidal \textit{B. thuringiensis} subsp. \textit{israelensis} (\textit{Bti}) and \textit{mogi} (\textit{Btm}), a vegetative cell growth was observed only in the \textit{Btm} culture 10 h post-incubation. It showed a possible recovery of vegetative \textit{Btm} cell growth compared to control without the supernatant. These results suggest that \textit{B. thuringiensis} subsp. \textit{cameroun} produced proteinous antimicrobial substances, one of which may be used as a selection marker to separate \textit{B. thuringiensis} subsp. \textit{mogi} after conjugating the two mosquitocidal strains.

Poster / Bacteria, Monday 16:00

\textbf{Effect of Bacillus thuringiensis berliner in combination with tannic acid and sodium bicarbonate on caterpillars of Galleria mellonella (greater wax moth)}

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In this study, caterpillars of Greater wax moth were collected out of the honey bees of west Azarbayzan and the they were cultured on old black wax for 1.5 years under 27 ± 2°C and 45 ±5 percentage relative humidity and different doses of three serotypes namely (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 probe analysis, their LC50 was calculated and after conjugating the two Kurstaki serotype with 415.049 pp.m LC50 with Spray Method and 734.26 ppm with powder method has had the best effect. In this study, tannic acid and baking soda (Sodium bicarbonate) in combination with low doses of Kurstaki Variety with Spray method were used as strengthening substance, the LC50 with probit analysis method and test with powder Method for tannic acid has been evaluated 22740.027 ppm and for baking soda 106,000,000 ppm. It was revealed that tannic acid with 12000 ppm which causes 48.9% death increases its killing property in combination with bacteria especially in 50,112,700ppm 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 instars 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 instars 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 accompanying 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 instars 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 tannic 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-mentioned substances are used on hives roofs in the from of mixture with ground wax.

Poster / Bacteria, Monday 16:00

\textbf{From non-target risk assessment to Bt resistance management: the example of Bt Brassica sp. and Pieridae}

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Insecticidal toxins produced by \textit{Bacillus thuringiensis} (\textit{Bt}) have been widely used in plant protection in two different ways: 1) by spraying bacterial \textit{Bt} formulations, 2) by expressing their gene encoding \textit{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 \textit{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 \textit{Bt}-transgene invasion. To assess the potential risk of \textit{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 \textit{Bt} crops and relative wild species and its hosts preferences. Moreover we report data on susceptibility to commercial \textit{Bt} and to \textit{Cry1Ab} toxins. 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

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

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\textit{Pseudomonas fluorescens} was isolated from Nile tilapia \textit{Oreochromis niloticus} fish. Three concentrations of \textit{4x10}^{11}, \textit{4x10}^{9} and \textit{4x10}^{7} cells/ml were used to study the pathogenicity of bacteria. The percentage of cumulative mortality in the \textit{O. niloticus} fingerlings was 76.70; 60 and 36.65%, respectively and the calculated LD₉₀ was 4 \textit{X}10⁶ cells/ml. Polymerase chain reaction and DNA probe were used for the effect of \textit{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 pattern in a 848 base pair lengths at different annealing temperature. DNA specific probe was tested with internal organs of diseased O. melilocus 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**

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*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 FS52 at 10, 15 and 20 °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 °C. Significant mycosis however was found to take place at the lower temperatures of 15 and 10 °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**

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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*10⁶ 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**

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*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 °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 5.57 ±10⁶ conidia.g⁻¹ on 14 September 2010 to 7.19 ×10⁶ 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* = 299.170.331.90 -5.692*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* = 299.170.331.90 -5.692*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* = 299.170.331.90 -5.692*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* = 299.170.331.90 -5.692*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* = 299.170.331.90 -5.692*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* = 299.170.331.90 -5.692*¹ on April 5 2011, a reduction of approximately 7.75 times the initial concentration.

**Poster / Microbial Control, Monday 16:00**  
**MC4**  
**Association of entomopathogenic fungi with exotic red palm weevil in treated and untreated Phoenix canariensis**

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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 monosporum* and *Trichosporon sp.*, *Verticillium lecanii* and *Verticillium* sp. (a member of the *Verticillium* lecanii species complex).

**20**
Molecular characterization and virulence of Beauveria isolates from emerald ash borer within old outbreak sites in Canada

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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, mycossed 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 habitants. 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 10^3 conidia/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 x 10^6, 2.0 x 10^5, 2.0 x 10^4, and 2.0 x 10^3 conidia/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 mycossed EAB than GHA suggesting that these local isolates have better qualities and can be used to manage EAB infestations.

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

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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 rDNA and partial β-tubulin gene sequences. Our phylogenetic analysis and constructed trees based on the SSU rDNA, ITS and tubulin genes clearly separate the Isaria and Paecilomyces into two distinct groups and explicitly confirm isolates L66B-A, SY17-A and LHV46-A as I. farinosa and B3A, B59A and SY458B as Paecilomyces lilacinus. Pathogenicity of the EAB-recovered I. farinosa and P. lilacinus was tested against EAB using a single concentration, 2.0 x 10^3 conidia/ml. Control isolates included three commercial isolates namely; Isaria (Paecilomyces) fumosoroseus (PPR-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.
in plots from the second area, and significantly more in the first (36.5%) and the third (57.5%) areas. Fungus Lsp 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 Pspp. There is a correlation between Bb and Lsp fungi: when the first fungus predominates, the second is seldom seen, and vice versa. For example, in case of the first area, 32 plots contained Lsp and only 4 plots contained Bb. A diametrically opposite situation was found in the third area where Bb was in 25 plots and Lsp 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 2x10³ and 6x10³, Ma between 2x10³ and 1x10⁴, Lsp between 2x10³ and 1x10⁴, Pspp between 2x10³ and 1.6x10⁴ and Ci between 4x10³ and 1.2x10⁴; the second area contained Bb propagules between 2x10³ and 2x10⁴, Ma 2x10³, Lsp between 1x10⁴ and 7x10³, and Pspp between 2x10³ and 3x10³; the third contained Bb propagules between 2x10³ and 2x10⁴, Ma 1x10⁴, Lsp 2x10⁴, and Pspp between 2x10⁴ and 1.6x10⁵. During the period of soil sampling there was no damage to sugar maple trees by pear thrips.

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

**Are Beauveria bassiana treatments appropriate for orchard canopies?**

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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 ≤ 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 x 10⁴ 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 x 10⁵ 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.

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

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

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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.

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

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

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The experiment was conducted to manage the stemborer, Chilo partellus Swinhoe by utilising 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 parasitization 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 spray 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 spraying 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**

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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 (LC50) of CpGV-C against third instar *C. pomonella* larvae was 7.7 × 10^5 OBs/ml at ninth day post inoculation. The LC50 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 1:1 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**

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Densoviruses (DNVs) are small icosahedral non-enveloped viruses, containing a single-stranded linear DNA genome, they belong to *Paroviridae* 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 & Mariain, 1975). We developed a new strategy for biological control based on the *Junonia coenia* Densovirus derived vectors. JcDNV has an ambisense 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 expressed under control of P9. Longer 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 produced by supernatant-junction 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 liturata*, Lep.: *Noctuidae*)**

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*Spodoptera liturata*, 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 numerous 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. liturata* 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 *Malacosoma neustria*) and FOTRB 11 (from *Balannius 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 1st, 2nd, 3rd and 4th 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. liturata* is planned.

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

**Preliminary results on antimicrobial activity of *Rhynchophorus ferrugineus* hemolymph**

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*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 4 structural 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 of entomopathogenic 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  
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Delta endotoxins are related to homologous polypeptides incoming in structure of parasporal bodies which are produced by numerous subspecies of Bacillus thuringiensis Berliner (Schneepf, et al. 1985). These types of toxins attract attention of researchers as very effective tools in 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 peroxidation 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 particular, delta-endotoxins take action on phytopathogenic fungi from different genera including Fusarium, Bipolaris, Phytophthora, Alternaria, Rhizoctonia and some others having significant importance for agriculture (Talpanova, 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  
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Bacillus thuringiensis (B. thuringiensis), is an 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 haemocoeil of insect is very important to the virulence of B. thuringiensis. The GntP (glucuronate permease) is responsible for the import of glucuronate, a screeningselective and 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 pLC333. 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 gene plays a more important role in the germination by providing the transport of glucuronate. The GntP was localized to the spor 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 Platella xyllostella susceptibility to Bacillus thuringiensis  
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Platella xyllostella (L.) is one of the most important pests of crucifers worldwide and Bacillus thuringiensis Berliner-based biopesticides are used as alternative to chemicals that may have undesirable effects on environmental. However, reports of rapid resistance evolution of P. xyllostella to B. thuringiensis based biopesticides were reported in some countries. A Brazilian population of P. xyllostella with 100 generations was assayed to B. thuringiensis isolates susceptibility. Biosays were performed with seven B. thuringiensis isolates cultivated in nutrient agar and incubated for 5 days at 30°C. Twenty six B. thuringiensis 0.5ml suspensions ranged from 9 x 10 to 3 x 10^3 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 LC50. Mortality was 100% to all isolates and data were adequate enough to estimate LC50 only to isolate T08.024 with an estimated value of 2 x 10^4 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  
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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 P04 from Phyllophthorus oblongus L., Ta16 strain from Tariaeaurrhynchites aequatus L. and Np1 strain from Neocoenorhinitius paucisau 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 microsympy 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 ssore-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 cosmetically sequencing and computational pipeline**

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Discovery new insecticidal crystal toxins 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 cosmetically 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 holotyope 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**

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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 *Platella 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 x 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 nymph period and life-span of *O. insidiosus* were affected by the consumption of eggs immersed in Agree®. *O. insidiosus* females were consumed *P. xylostella* eggs that were immersed in the product Agree® had lower number of offsprings, thereby resulting in a lower population growth rate.

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

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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 (*Platella 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 water, (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.

**Interaction between Bacillus thuringiensis and Telenomus remus**

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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 were 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 bioassay was conducted similarly but using deionized autoclaved water. The evaluated parameters were parasitism, sex ratio, viability of parasitoid 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 entomopathogenic 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
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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 ± 1° C, relative humidity of 70 ± 10% and photophase of 12 hours. The Dipel® was dissolved according to the manufacturer’s recommendations in humidity of 70 ± 10% and photophase of 12 hours. The Dipel® was 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 concentration in leaves of crucifers unsprayed 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.
Panulirus argus e-7.4, s processed for histology and electron microscopy to investigate the phylogenetic comparison with WSSV, sampling juvenile WSSV is important. We attempted to rediscover B virus to enable within European waters of a pathogen that mimics the pathology recently been listed in EC Directive 2006/88, the historic presence on Taxonomy of Viruses (Vlak classified within the White Spot Syndrome Virus (WSSV), it has been tentatively approached, due to its morphological and pathological similarity to Bacillus thuringiensis (B virus/WSSV in crabs. Further phylogenetic work is underway to characterise HLV. The search for B virus continues.

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 ×10³ 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 (suspending). 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.

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 eosinophilic 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.

White Spot Disease mimics: hunt for the elusive B virus in European waters of a pathogen that mimics the pathology recently been listed in EC Directive 2006/88, the historic presence on Taxonomy of Viruses (Vlak classified within the White Spot Syndrome Virus (WSSV), it has been tentatively approached, due to its morphological and pathological similarity to Bacillus thuringiensis. 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 ×10³ 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 (suspending). 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.

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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.

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

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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 six plus stranded 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.

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

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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 positive for 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.

Identification of first toxins of Paenibacillus larvae

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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 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 Ptx1, Ptx2 and Ptx3. 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.

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

Chris Hauton1; Meggie Hudspith1; Richard Edwards1; Tim Elliott1
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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 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
Unravelling the function of secondary metabolites of *Paenibacillus larvae*

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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 analyses 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.

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

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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 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.

Potential siderophore in *Paenibacillus larvae*

Gillian L Hertlein; Eva Garcia-Gonzalez; Lena Poppinga; Anne Fünhau; Elke Genersch

Institute for Bee Research, Friedrich-Engels-Str. 32, 16540 Hohen Neuendorf, Germany.

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 bacterium *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 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 micronutrients 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.
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, spor 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. 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***

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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 attracted 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 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-knoutant 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***

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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 of the krill can 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

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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 low 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 microscopic 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 (Crassostrea virginica) hemocytes following administration of β-glucans**

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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 hemelymph 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 marine 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

Extreme dimorphism in a microsporidian infecting the musculature of marine crabs

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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 microsporidian from marine crustacean hosts.

Virus Division Workshop Presentation, Monday 21:00

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

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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 come 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?
Model systems in general use familiar animals and plants with well-known life histories to ask questions about how the world works. Behavioral ecology is a good example 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.

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 this pest 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.

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Contributed Papers  Tuesday, 9:15-10:00

Nematodes 1

Contributed Paper. Tuesday 9:15

Host-parasite interactions between mermithid nematodes and mosquitoes

Randy Gaugler1, M.M. Shamseldin1, Yi Wang2

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The mermithid nematodes Romanormes iyengari and Sterklavormis 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 p. 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 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

Investigations into a nematode infecting an exotic invasive ant: new host relationship or hitchhiker across the Atlantic? 
Eleanor Groden1, S. Patricia Stock2, Robert Lopez3, and Jennifer Lud2

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

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Myrmina 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 diplodogaster-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 Diplodogasteridae 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

Scavenger Deterrent Factor (SDF) from Symbiotic Bacteria of Entomopathogenic Nematodes
Selcuk Hazir1, Baris Gulcu2 and Harry K. Kaya1

1 Adnan Menderes University, Faculty of Arts and Sciences, Department of Biology, Aydin, Turkey; 2 Duze University, Faculty of Arts and Sciences, Department of Biology, Duze, Turkey; 3 Department of Nematology, University of California, Davis, CA 95616

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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 marcescens, 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 Paper, Tuesday 8:00

IE0 coupled with low levels of IE1 enables rapid DNA replication in Autographa californiae multiple nucleopolyhedrovirus (AcMNPV): Rationale for the requirement of IE0 and IE1 to achieve wildtype infection
Nadia R. Sokol1, Yingchao Nie1; Leslie G. Willis2; Mark Rheault3; David A. Theilmann4

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

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The exact functions of the two primary trans-regulatory proteins, IE0 and IE1, in baculovirus 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, or ie1. Expression of ie0 results in the translation of both IE0 and IE1. In addition, IE0 is produced. Viruses were constructed that expressed ie0, ie0, 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 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.

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 Nie1, Minggang Fang2, Martin A. Erlanson3; David A. Theilmann4

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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-ac68KO), which was repaired with just ac68, lef-3 or both lef-3 and ac68, to generate lef3-3KO, ac68KO or the lef3-ac68 double repair virus, respectively. To produce the ac68KO without any potential impact on lef-3, the lef3-ac68KO 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 lef3-3KO. The ac68KO 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.

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

Contributed Paper, Tuesday 9:15

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

Guozhong Feng1, David Thumb2, Basil Arif1, Daniel Douce1 and Peter J. Krell

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*Autographa californica* multiple nucleopolyhedroviruses (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 transposoning 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

Construction and characterization of a recombinant AcMNPV with broader host range

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

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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 S99 cells co-infected with a pB5-null AcMNPV mutant and partial DNA fragment of SmMNPV. vAcRev can replicate not only in parental virus permissive cell line, such as S99, H5 and Sc301, 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.
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 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 lentil fields 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 flatfan 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 (*Trichomallocis surcophagae*), crayfish resident in southern Alberta, a crustacean (*Ceriodaphnia dubia*) and earthworms indicated no adverse non-target effects.

**Contributed Paper, Tuesday 8:30**

**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. Maniania¹, P. Nana¹, H.I. Boga², P. Kamthoung³

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Spot-spray application of *Metarhizium anisopliae* formulated in oil extract of *Calpurnia aurea* was evaluated for attraction, infection and dissemination of inoculum among adult *Rhipicephalus appendiculatus* ticks in semi-field experiments. Formulation was sprayed on 900-cm² 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. Most 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**

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

Sunday Ekesi and Nguyu K. Maniania

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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 10¹² 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**

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

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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 LC₅₀ for adult thrips was between 10⁻³ - 10⁻⁴ 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 (SufOil-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, SufOil-X and spinosad treatments at the end.
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 (BotanGard), 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.

Symposium Paper, Tuesday 9:45

Developing functional biopesticides for the lawn care industry

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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; Millennium®) 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 granular 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 Paper, Tuesday 10:30

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

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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 anecstral 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 an unusual virus in the Family Totiviridae. Its closest relatives infect yeast and protozoan parasites including Saccharomyces cerevisiae, Giardia lambia and Leishmania spp. IMNV has a double-stranded RNA genome of 7560 bp. The virion is isosahedral 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 hemocytes infiltration and fibrosis. Significant lymphoid organ spheroid formation is typically present, and ectopic lymphoid organ spheroids are often found in the hemocoele and loose connective tissues, especially in the heart lumen and adjacent to arterial 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
Evolutionary epidemiology of viral pathogens in shrimp aquaculture

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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
Molecular epidemiology of white spot syndrome virus

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

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It has been hypothesized that random viral inserts occur autonomously in shrimp and lead to specific, heritable antiviral immunity. To test the prediction regarding random viral inserts, giant tiger shrimp specimens were examined for random genomic insertions of the dsenovirus HHHNV using PCR analysis with a set of 7 overlapping primer pairs to cover the whole HHHNV genome (~4 kb). PCR failures revealed a high proportion of specimens with...
random sequence gaps, suggesting the presence of IHNV 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 microsatellite-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 IHNV 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 IHNV 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 occurs in the shrimp genome. These inserts can give false positive results for infectious IHNV 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 Jarosnki

Symposium Paper, Tuesday 10:30
Introduction: What is IPM anyway?
S. Jarosnki (USDA ARS, Sidney MT)

Symposium Paper, Tuesday 10:45
Microbial Pest Control Agents in Orchard IPM
Lawrence A. Lacey
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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 incoherently 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
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
Microbial Control Agents in Greenhouse IPM
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Several factors prompted a major shift in greenhouse vegetable 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 integration 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
Entomopathogens in small fruit IPM
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There are several studies demonstrating the potential of entomopathogens as control agents for a number 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**

**A multi-gene analysis of the microsporidia as eukaryotes**

Charles R. Vossbrinck, Heng Xiang

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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 Microsporidia 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 Microsporidia as distant (but perhaps not distinctly 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**

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

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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 microsporidians 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 SSU/rRNA of N. bombycis, PCR primers for detection of most insect microsporidians 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-1α genes of N. bombycis from GuangXi province isolate, N. Antheraeae from Shandong province, microsporidian spores from mulberry looper (Hemorophila atrilimeata) isolate (SCH), and from cabbage butterfly (Pieris spp.) isolate (CFD), and from diamondback moth (Plutella xylostella) isolate (XCE), respectively. The pseudogenes of SSU DNA of N. bombycis (GX), SCH, CFD were separately amplified 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**

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

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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 tubule 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**

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

Toby J. Wilkinson; Jenny Rock; Nia M. Whiteley; Mykola O. Ovcharenko; Joseph E. Ironside

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Microsporidia of the genus Dicytocoela 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.
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 binding and fusion was identified in BV envelope. Apart from the above, other proteins including some baculoviral 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 in 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.

**Roles of microRNAs in regulating host-virus interactions**

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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.
then allow AcMNPV spread into the hemocoel. Sindbis viruses do not encode gfp 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 Paper, Wednesday 8:30**

**Microsporidian-Induced Effects on the Host**
Organizers: Dörte Goertz

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

Raquel Martín-Hernández1; Aránzazu Meana2; Neil Boonham3; Mariano Higes1

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Nosemosis is the most widespread of adult bee diseases and causes economic losses to beekeepers (OEI, 2008; Giersch et al., 2009; Heinitz 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, BACTINE 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**

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

Cédric Alaix1; Claudia Dussaubat1; Jean-Luc Brunet1; Cynthia McDonnell2; Raquel Martín-Hernandez2; Mariano Higes2; Luc Belzunce1; Yves Le Conte1

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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 affects pheromone synthesis of both workers and the queen. Altogether those data show that besides weakening bees at the individual level, *N. ceranae* can also disturb the colony homeostasis and provide important insight into honey bee/microsporidian interactions.

**Symposium Paper, Wednesday 9:30**

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

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Infected locusts with *Paras nosoema 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 unfected 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 1×10³ spores/ml, locusts had F/C ratios that were significantly (P<0.05) more solitaria than untreated locusts, with locusts having 1×10⁻⁵ spores/mL, locusts had F/C ratios that were significantly higher at day 16, female locusts were transiens at higher 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 8:00**

**Bacteria 2**

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

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*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 identified. 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. Analysis of the expressed cadherin proteins showed a molecular weight of 55 kDa, which correponds to the predicted molecular weight of the cloned APNs which were located in the membrane fraction. Four out of the five expressed proteins showed a molecular weight of 55 kDa. 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 sensitivity to inhibitors (bestatin, actinonin and EDTA). The APN activity to Cry1Ac was significantly reduced, suggesting that the APNs expressed in the *O. nubilalis* midgut are not functionally redundant.  

**Contributed Paper, Wednesday 8:15**  
**90 STU**  
**Characterization of the Cry1Ac-induced midgut regenerative response to intoxication in *H. virescens* larvae**  
Anaïs S. Castagnola1, Omalithage P. Perera2, Juan Luis Jurat-Fuentes3  
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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. Craya, Yolanda Bel, Agata Jakubowska, Baltasar Escrú

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The lepidopteran aminopeptidases (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 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. Analysis of the expressed cadherin proteins showed a molecular weight of 55 kDa, which correponds to the predicted molecular weight of the cloned APNs which were located in the membrane fraction. Four out of the five expressed proteins showed a molecular weight of 55 kDa. 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 sensitivity to inhibitors (bestatin, actinonin and EDTA). The APN activity to Cry1Ac was significantly reduced, suggesting that the APNs expressed in the *O. nubilalis* midgut are not functionally redundant.

**Contributed Paper, Wednesday 8:45**  
**92 STU**  
**Quantitative Cry1Fa toxin binding analyses using indirect radiolabeling**  
Siva R. K. Jakka1, Joel Sheets2, Ken Narva3, and Juan L. Jurat-Fuentes4  
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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 Palma1, C. Sara Hernández-Rodríguez2, Mireya Maeztu2, Patricia Hernández-Martínez2, Ilígo Ruiz de Escudero1, Baltasar Escrú,1 Delia Muñoz2, Juan Férez2, Primitivo Caballero2

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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 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
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 RFLP method to condense the Bacillus thuringiensis collections. 

The salivary secretome of salivary gland hypertrophy virus-infected tsetse fly Glossina pallidipes (Diptera: Glossinidae) was studied using PCR based technique. Twenty-six isolates identified to the subspecies were used: 9 strains from the USDA, 9 from Institut Pasteur and 8 from EMBRAPA, and the other 39 isolates without identification of subspecies also belonging to EMBRAPA were subjected to RFLP method to condense the Bacillus thuringiensis collections. 

The salivary secretome of salivary gland hypertrophy virus-infected tsetse fly Glossina pallidipes (Diptera: Glossinidae) was studied using PCR based technique. Twenty-six isolates identified to the subspecies were used: 9 strains from the USDA, 9 from Institut Pasteur and 8 from EMBRAPA, and the other 39 isolates without identification of subspecies also belonging to EMBRAPA were subjected to RFLP method to condense the Bacillus thuringiensis collections.
(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) of salivary fluid proteins was 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,1 2 Henry Karithi,2 Andrew G. Parker,2 Bryony C. Bonning,2 Just Vlak,2 Max Bergoin,1 and Marc J.B. Veyssen3
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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 meals 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 Etebāri, Robin Palfreyman, David Schlipalius, Lars Nielsen, Richard V. Glaz and Sassan Asgari
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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 and 10000 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. Diadegma semiclausum symbiotic polydnaviruses, known as ichnoviruses, play significant roles in host immune suppression and developmental regulation. In the current study, D. semiclausum ichnovirus (DvIV) genes expressed in the host were detected and 19 unique sequences identified from five PDV gene families including vankynir, viral innexin, repeat elements, a cysteine rich motif, and polar residue rich protein. Vankynir 1 and repeat element 1 genes showed the highest transcription levels among the DvIV genes.

Contributed Paper, Wednesday 11:15 100 STU Toward understanding of bacovirus mechanism replication

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

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A lot of parasitic wasps carry in their genome bacoviruses, which replicate in the wasp ovaries to formed viral particles. These particles packaged several dsDNA circles and are injected during oviposition in the haemoceol 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 bacovirus does not replicate in the parasitized host. We can considered that the bacovirus 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 bacoviral/ nudiviral origin of bacoviruses (Bezier et al, Science 2009), and the sequence of the integrated form of some bacoviruses offers us for the first time the
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 ankyrin 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 ankyrins 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 ankyrin genes were compared between S. frugiperda and S. littoralis and in different insect cell lines. Our results showed that, among all ankyrin 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

A host translation inhibitory factor of Cotesia plutella bracovirus discriminates mRNAs depending on its dependency on eIF4A

Yongyeun Kim, Surakasi V. Prasad, Rahul P. Hepat
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An endoparasitoid wasp, Cotesia plutella, depends on its symbiotic Polydnavirus, C. plutella 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 successfully. 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 randomly chosen in terms of 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-down 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

The evolution of the largest gene family of bracoviruses

Céline Serbielle1, Stéphane Dupas2, Elifie Perderau3, François Héricourt1, Elisabeth Huguet1, Jean-Michel Drezen1
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Gene duplications have been 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

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

Shu Zhang1, Kavita Bitra1, Markus H. Beck2
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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 Klingens

Symposium Paper, Wednesday 10:30  
From basic pathology to microbial control: on overview of fungal acaropathogens
Leo P.S. van der Geest
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The Kingdom Fungi is a monophyletic assemblage with four phyla: Chytridiomycota, Ascomycota, Basidiomycota and 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 known sexual stage. Chytridiomycota represent a primitive group of aquatic fungi with no species that infect mites. The order Entomophthorales (Zygomyota) contains several species (Neocygites 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 Phyllocoptra 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  
Fungal control of ticks in Europe: Methods and first results
Gernot Walder1, Hermann Strasser2, Angelika Prader1, Roland Zelger2
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Human and animals are under increasing threat from ticks of the Ixodes ricinus complex that have proven 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  
Fungal control of ticks in South America
Everton K. K. Fernandes1,2, Donald W. Roberts3 and Vânia R. E. P. Bittencourt
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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  
Research priorities for development of effective biological pesticides for control of cattle ticks
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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;
The two spotted spider mite Tetramychus urticae is known to overwinter as hibernating females, and these partly inactive females may harbour Neozygites floridana. N. floridana is a 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 -15.3°C). 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.; Vitalis Wafala Wekesa; Karin Westrum; Vanessa da Silveira Duarte; Thiago Rodrigues de Castro; Silje Stenstad Nilssen; Ingeborg Klingen
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The mite-pathogenic fungus Neozygites floridana and N. tanaeae 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, rainfall does 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.

Symposium Paper, Wednesday 11:50 109
Overwintering of Neozygites floridana and its importance in conservational biological control of spider mites
I. Klingen1; G. Waersted2 & K. Westrum1
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The two spotted spider mite Tetramychus urticae is known to overwinter as hibernating females, and these partly inactive females may harbour Neozygites floridana. N. floridana is a fungal pathogen on T. urticae. Hibernation of T. urticae females 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.

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)
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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. viridans’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.
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 in 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 present 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.

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**Contributed Paper, Wednesday 11:00**

**113 STU**

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

**Daniel Hines**1,2; Adam R. Acorn1; K. Fraser Clark1,2; Rémy Rochette3; M. John Tremblay1

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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.

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**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. Meres1**, Cyril C. Ajuzie2, Masi M. Sikaroodi3, Jeffrey D. Shields1, Patrick M. Gillevet1

1Environmental Science and Policy Department, George Mason University, 4400 University Drive, Fairfax, VA, 22030, 2Aquaculture, Freshwater and Marine Ecology Research Lab, Applied Fisheries and Hydrobiology Unit, Department of Zoology, University of Jos, Nigeria, 3Virginia 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.

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

Honouring Lerry Lacey and Harry Kaya

Organizers: Steven Arthurs and Ed Lewis

**115**

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

**Steven Arthurs**

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**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

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

Brian A. Federici
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Symposium Paper, Wednesday 15:00

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

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

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

Katherine A. Aronstein 1, Scott Cormack 2, Anna Bennett 3

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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 Ascosphaera 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

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

Francis Drummond 1, Katherine Aronstein 2, Brian Eitzner 3, James Ellis 4, Jay Evans 5, Nancy Ostiguy 6

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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 each. The project started in April 2009 and 2010 from 3 lb packages and queen 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 conducted. 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.
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 proteomic and histological evidence of the presence of octopamine receptors in cricket hemocytes. Immunohistochemistry 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.

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 debilitating state. Sickness behaviour is well characterized in vertebrates, but studies in 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.

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 enhancer and a chitinase-like protein. Knowing that enhancers 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 chitin-binding proteins during P. larvae infection.

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. Easby & Shelley A. Adamo
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Contributed Paper, Wednesday 14:45 123 STU Sickness behaviour in the Texas field cricket Gryllus texensis
Evan R. Fairn; Shelley A. Adamo
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Contributed Paper, Wednesday 15:00 124 STU Chitin-binding proteins of Paenibacillus larvae and their role in pathogenesis
E. Garcia-Gonzalez; L. Poppinger; E. Genersch
Länderinstitut für Bienenkunde, Molekularle Mikrobiologie und Bienenkrankheiten, 16540 Hohen Neuendorf
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Contributed Paper, Wednesday 15:15 125 Infection parameters for Nosema ceranae and Nosema apis in Apis mellifera
Wei-Fone Huang; Yanping Chen; Leellen F. Soltner
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;
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Contributed Paper, Wednesday 15:30 126 STU The expression strategy of the Acheta domestica densovirus (AdDNV) capsid protein (VP) gene cassette is so far unique among paroviruses
Kaiyu Liu; Yi Li; Françoise-Xavière-Jousset; Qing Yuan; Hanh Thi Pham; Jozsef Szelei; François Lépine; Max Bengoin; Peter Tijsen
1 INRS-Institut Armand-Frappier, Université du Québec, Laval, QC, Canada; 2 currently in Central China Normal University, Wuhan 430079 People’s Republic of China; 3 Université Montpellier2, Montpellier, 34095 France.
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Recently, the cricket industry in North America, supplying food for reptile pets in 6-million American households, has been devastated by AdDVN epizootics (Szeli 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 (1a 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 and define a new genus of ambisense denvirovirus. X-ray crystallography of the capsids of this virus is underway.
3D structure of a novel bacterial toxin complex isolated from *Yersinia entomophaga* MH96 and implications for insecticidal activity

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*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 PA1H6. Residing within PA1H6 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

Electron-microscopic and genetic characterization of a *Rickettsiella* sp. infecting the manuka beetle, *Pyronota setosa* (Coleoptera: Scarabaeidae)

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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, as whitened larvae of one of the outbreak species, *Pyronota setosa*. Microbial diagnosis indicated an intracellular, 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*. 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

The grape phylloxera and *Pantoea agglomerans* – a perfect match?

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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 *Xenorhabdus* 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 (10³ spores/mL) or a high (10⁵ 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 reproducibility 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

* Bacillus thuringiensis* delta-endotoxins activity against nematodes

Tatiana A. Malinina 1, Ludmila K. Kameńek 1, Valery M. Kameńek 2, Maxim A. Terpilovsky 3

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Phytoparasitic nematodes cause severe crop damages annually, with losses reaching 90%. In our studies, the free-living nematodes *Turbinifer 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 to be highly effective and safe to human microbial control agent, active against phytopathogenic microorganisms.
Monalysin, a novel β-pore-forming toxin produced by the entomopathogenic bacterium Pseudomonas entomophthali.

Onya Opota 1, Isabelle Vallet-Gélé1,2, Renuad Vincentelli1, Christine Kellenberger1, Ioan Iacovache1, Manuel Rodrigou Gonzales1, Alain Roussel1, Françoise-Gisou van der Goot1, Bruno Lemaître1.

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Pseudomonas entomophthali is a recently identified bacterium that is able to infect and kill Drosophila as well as insects from different orders. Ingestion of P. entomophthali infects 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. entomophthali 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 entomophthali 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 Pvs (Pseudomonas 2 Virulence Factor), a secondary metabolite biosynthesized 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. entomophthali. Here, we will describe the properties of this new pore-forming toxin and its contribution to P. entomophthali virulence towards insects.

Contribution Paper, Wednesday 18:15

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. Steinwender1, Jürg Enkerli2; Franco Widmer2; Jürgen Eilenberg1; Nicolai V. Meyling1

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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 52 plots) in Denmark were isolated, using Tenebrion 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 (86.5%), M. robertsi (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 grasshopper

Stefan T. Jaronksi and John A. Gaskin

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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 Boc 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 six 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 Boc haplotypes we observed both morphological differences and oosporein production differences, indicating more genetic diversity than indicated by the Boc region. Higher resolution analyses are being conducted.

Poster / Fungi, Wednesday 16:00

F3 STU

Phylogenetic and pathogenic divergence within Metarhizium majus lineage

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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 Protoetia orientalis (Scarabaeinae, 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. (Scarabaeinae, Rutelinae) and one isolates from soil was close to the ones from Orcestes sp. (Scarabaeinae, 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 Scaraeidae species might have happened within M. majus clade.

Poster / Fungi, Wednesday 16:00 F4 Biological control of Rhizipehalus microplus: An intensive search for promising fungal biological control agents Everton K. K. Fernandes1,2, Isabele C. Angelo1, Drauzo E. N. Rangel1, Thiago C. Banihense1, Asrea M. L. Moraes1, Donald W. Roberts2 and Vânia R. E. P. Bittencourt1 1Curso de Pós Graduação em Ciências Veterinárias, Universidade Federal Rural do Rio de Janeiro, Seropédica, RJ, Brazil; 2Department of Biology, Utah State University, Logan, UT, USA; 1Laboratório de Taxonomia, Bioquímica e Bioprospecção de Fungos, Instituto Oswaldo Cruz, Fundação Oswaldo Cruz, Rio de Janeiro, RJ, Brazil; *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, *Rhizipehalus 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 481, CG 484, 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 LC50 between 103 and 104 conidia ml-1; produced 5,000 conidia or more on 60 mm2 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, single 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 suchlaspera* 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*.

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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.-Crv) 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 of and the reconstituted pheromone blend on the in vitro germination and vegetative growth of two isolates of *B. bassiana* 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)

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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 increased in 10 days (130.1–216.3 aphid/plant). In parasitoid treated with *Lecanicillium* sp. 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 + fungit 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.

**Fungi**

**F8** Biological evaluation of *Cordyceps militaris* InPyo Hong, 
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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 led to the isolation of cordycepin, guanosine and tryptophan. All these three compounds showed significant inhibitory activity on adipocyte differentiation. Further studies with different isolates of *C. militaris* have been carried out in the current study. 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 led to the isolation of cordycepin, guanosine and tryptophan. All these three compounds showed significant inhibitory activity on adipocyte differentiation. Further studies with different isolates of *C. militaris* have been carried out in the current study.

**F9 STU** Evaluation of entomopathogenic fungus *Lecanicillium muscarium* hybrid strain 2aF43 formulation as biological control agent of greenhouse whitefly, *Trialeurodes vaporariorum*

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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 1.7×105 spore/ml and 3.0×109 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 69.0 cfu/cm2, 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.

**F10** UV-B and heat induced post-stress growth delay

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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* spp were exposed to 45ºC heat for 5 hours, 17 other isolates were exposed to UV-B radiation for 4 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 delay (PSGD) was DWR 1280, a *M. acridum* isolate known to have high tolerance to heat. Of the 17 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 hours after treatment and 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
infective 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**

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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 *Metharhizium robertsi* 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. robertsi* isolate. All *Tolypocladium* isolates, however, were less tolerant to UV-B radiation than the isolate of *M. robertsi* 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. robertsi*. 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. robertsi* 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. robertsi* 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. robertsi* 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 *Metcariuzium anisopliae***

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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, *Metcariuzium 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 Hecloverapa. 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 *T. 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***

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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. Among 37 isolates, 13 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**

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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 farinosus 1 P. lilacinus 14 Lecanicillium araneicola, 94 Lecanicillium spp., 8 Sip模拟被ans laleni와 1 S. lasononivem were obtained from Japanese mosquitoes, and 3 B. bassiana, 1 I. farinosa, 77 L. araneicola and 13 S. lasononivum 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 L. 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 lady beetle, Adalia bipunctata L. (Coleoptera: Coccinellidae)

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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 Tabulinoessa 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 unidentified microsporidium-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

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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 non-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

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Nosema ceranae is an obligate, intracellular microsporidium infecting the honey bee (Apis mellifera) midgit. 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 midgit 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 NaHCO3 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.
**Microsporidia in migratory beekeeping in the United States**

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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.

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**An investigation on efficacy of entomopathogenic nematodes on leopard moth, Zeuzera pyrina L. (Lep.: Cossidae) in Iran**

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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 carpocapsae* were evaluated via laboratory assays on different larval stages of this insect. Also susceptibility of the *Z. pyrina* larvae to *H. bacteriophora* and *S. carpocapsae* 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 vs. 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 almond 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.
Factors affecting hatching pattern of the eggs of *Steinernema carpocapsae* (Nematoda: Mermithidae)

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We assessed the number of *Steinernema carpocapsae* Poinar and Camino, 1986 preparasites obtained from an initial known number of nematode eggs and how some abiotic factors (temperature-photoperiod, flooding-dissection) could affect the number of emerged preparasites. Two groups of eggs, one maintained flooded and other under different number of flooding-dissection events (each 15, 30 and 60 days), were performed. Each group of eggs was also studied under two conditions: 25°C and 14:12 (L:D) photoperiod, and at 16°C and 12:12 (L:D) photoperiod. When eggs were maintained flooded a higher total percentage of *S. carpocapsae* preparasites was reported compared to treatments subject to drying-flooding cycles. The maximum percent of J2s emerged was found in the treatments maintained flooded at 16°C and 12:12 (L:D) photoperiod (30 ± 15.04%). Preparasites were observed from 7 (25°C) and 14 (16°C) days suggesting this period as the minimum 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 25°C and 16°C, respectively) suggested a nonsynchronous hatching of nematode eggs possibly due to non uniform embryonation of eggs. The different periods of exposure of the assays to drought conditions did not affected significantly the total average percentage of J2s emerged; however a nonsynchronous hatching of nematode eggs possibly due to non uniform embryonation of eggs was suggested.

Pathogenicity, Biology and Production of a new isolate of *Heterorhabditis bacteriophora* (Poinar, 1976) (Nematoda: Heterorhabditidae) from Argentina

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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 15:1 IJs/larva in Petri dishes (10 cm diameter) at 25°C. Six replicates 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 replicates, parasitized cadavers were collected and 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 12th. 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 *Lobiola insularis* (Coleoptera), an important emerging pest of strawberry crops in Argentina.
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**

**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**

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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, dpnpol, vp28), immune (carrinin, omp, peroxinectin, prophenoloxidase) and endogenous reference genes (D-actin, ubiquitin, tubulin, gapdh, ef1a 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**

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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 (ObrNPV) 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 ObrNPV isolates from British Columbia, Canada, Norway the United Kingdom, and Massachusetts, USA. A phylogenetic analysis of these sequence data indicated that the ObrNPV 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**

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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**

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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-9, 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/HaSNPV 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 (orf50orf5b, hr3orf62, orf26, orf79, orf12orf117a, 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/HeSNPV and He/HzSNPV isolates. Gemstar and two HeSNPV isolates exhibited significantly higher LC50s than the Hz/HeSNPV isolates tested. This study expands significantly on what we know about the variation of heliothene NPV populations and provides novel information on the distinct groups in which these NPVs occur.

The polyhedrin is responsible for forming polyhedra of nucleopolyhedrovirus (NPV) and highly conserved in most completely sequenced 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 exigua* 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.

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 vivo* 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 *vivo* production. In previous studies, six Many Polyhedra (MP) variants were selected after seven successive passages of the *Anticarsia gemmatalis* nucleopolyhedrovirus (AgMPNV-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 AgMPNV-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.
AgMNPV (Anticarsia gemmatalis Nucleopolyhedrovirus), the most widely used viral biopesticide, 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 in the virus genome by Pyenotes 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 biostematical activity.

Poster / Viruses, Wednesday 16:00

Comparative analysis of mononucleotide repeat (MNR) sequences in the genomes of baculoviruses

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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 mononucleotide repeat (MNR) sequences in the genomes of baculoviruses. One of the viruses, Pyenotes tritici exhibits an increased biostematical activity.

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

Identification of soybean aphid viruses using Next Generation sequencing technology

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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 Euproctis chrysorrhoea virus (EeV: Tettaviridae) 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-out aphid, Rhopalosiphum padi (Linnaeus) and the green peach aphid, Myzus persicae (Sulzer).
A species complex of climbing cutworms, mainly in the genera Euxoa and Abagrotis, are pests of grape vineyards in the Okanagan region of Canada. A series of Alphabaculovirus strains were isolated from single-infected-larva samples from cutworm populations in vineyards across the Okanagan 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 158 ORFs and contained eight homologous repeat regions. This analysis indicated that the Abagrotis reedi nucleopolyhedrovirus (AbreNPV) ORFs typically had the highest homology to Agrotis ipsilon NPV (AgiNPV) ORFs. In addition the AbreNPV genome was largely collinear with that of AgiNPV. 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  
**V13** Genome structure of a nucleopolyhedrovirus infecting Abagrotis reedi, a cutworm pest of vineyards.  
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A species complex of climbing cutworms, mainly in the genera Euxoa and Abagrotis, are pests of grape vineyards in the Okanagan region of Canada. A series of Alphabaculovirus strains were isolated from single-infected-larva samples from cutworm populations in vineyards across the Okanagan 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 158 ORFs and contained eight homologous repeat regions. This analysis indicated that the Abagrotis reedi nucleopolyhedrovirus (AbreNPV) ORFs typically had the highest homology to Agrotis ipsilon NPV (AgiNPV) ORFs. In addition the AbreNPV genome was largely collinear with that of AgiNPV. 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)  
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The larvae of Neodiprion abietis (Har.) are 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 this 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, QTOF 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, P69 and ODV-E18. In addition, nine novel proteins were discovered encoded by NEAB13 (81 kDa), NEAB20 (20.0 kDa), NEAB33 (12.0 kDa), NEAB45 (22.0 kDa), NEAB70 (7.0 kDa), NEAB75 (10.9 kDa), NEAB82 (18.0 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 (NesNPV), 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)  
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The Nicaraguan population of Spodoptera frugiperda multiple nucleopolyhedrovirus, SIMNPV-NIC, is structured as a mixture of genotypes. SIMNPV-C, -D and -G pure genotypes are incapable of oral transmission. In SIMNPV-D and -G, the non-transmissible phenotype is due to the absence of pif1 and pif2 genes. The complete sequence of the SIMNPV-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 SIMNPV-G genome compared with that of SIMNPV-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 SIMNPV-B genotype in mixtures with SIMNPV-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  
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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 tested 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

Characterization of new active transposons isolated from insects


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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 baculovirus as 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, H15 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

Construction of the full-length cDNA clone of Ectropis oblique picorna-like virus

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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 391-9931) 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 Iflavirivirus 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

Study of late expression factors of Spodoptera frugiperda nucleopolyhedrovirus in the permissive insect cell line SF-9

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Baculovirus expression of late genes depends on the activity of a group of genes, collectively known as late expression factors (lef), that includes components of the DNA replication machinery and the viral RNA polymerase complex. Lef are been more extensively studied in the model virus AcMNPV, a group I alphabaculovirus. Nineteen AcMNPV lef 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 lef in other baculovirus lineages it is possible that functional homologs occur in other species. We are interested in studying lef of SIMNPV, a group II alphabaculovirus. SIMNPV infects productively SF-21 and SF-9 cell lines, as AcMNPV does. However, SIMNPV lacks homologs of AcMNPV lef in-2, lef-12 and p35. We constructed a SIMNPV 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 SIMNPV lef were not functional in this assay; therefore, in order to identify putative genes able to complement

Poster / Viruses, Wednesday 16:00

Construction of novel baculovirus expression vector system by fusion of partial polyhedrin

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Polyhedrin is the major component of the nuclear viral occlusion 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.
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 left 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.

Sf-caspace-1 is the principal effector caspase in Spodoptera frugiperda cells. Like the caspases in other organisms, Sf-caspace-1 is processed by upstream caspases to form an active heterotrimer that is composed of the p19 and p12 subunits. In mammalian 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-caspace-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-caspace-1 polypeptides: the pro-Sf-caspace-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-caspace-1 were also unstable and degraded by ubiquitin-proteasome system.

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 polyhedrin 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 (vAcA33), an ORF33 insertionional mutation, the upstream ORF32 (vFGF) with an intentional 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.

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 findings for the safe use of baculovirus in in vivo studies are discussed.
Many endoparasitic wasps inject a polydnavirus into their caterpillar hosts during oviposition. The viral entities associated with these wasp 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 1° instar *Chortoneura 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.

**Cross Divisional Workshop**

**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**

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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, baculoviruses, *Autographa californica* nuclear polyhedrosis virus (AcNPV) and *Bombyx mori* nuclear polyhedrosis virus (BmNPV), resulted that the AcNPV protein Polyhedrin increased comparing native form of gB, gC and gD. Fusion expression of ADV glycoproteins with partial polyhedrin increased comparing native form of gB, gC and gD.
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 pyramid-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

Pyramided crops incorporating RNAi technology

William Moar; Tom Clark; Parthasarathy Ramaseshadi; Gerrit Segers; Graham Head.
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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

Successes and challenges in commercialization of micro- and macro-biologicals

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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; Nemasyss®] 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; Trioche 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

Novel targeted delivery systems in biocontrol

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RNAi products platform for invertebrates’ health and Targeted Pest Control

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Ribonucleic Acid interference (RNAi) applications for invertebrates are on the scientific agenda since RNAi was initially introduced. The work done on c-elegans 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 aspects 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

Integration of Entomopathogenic Fungi into Insecticide Resistance Management Programs for Control of Sucking Insect Pests

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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 coincidently 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**

**Novel microbial controls from the Enterobacteriaceae**

Mark Hurst

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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 xylostella*). 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.
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.

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 mycelized 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.

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 (99%), 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 difference of 25% 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 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.
Evaluation of Fusarium coccophilum as a biological control option for armoured scale insects

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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 phytotoxicity, 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 Hemeroblesia latuanae (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 LT50 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 to 75% insect mortality resulted when scale infested Actinidia delicosa (A. Chev.) C.F. Laing & A.R. Ferguson ‘Hayward’) vines were sprayed with an aqueous formulation (4x105 conidia/mL). No phytotoxicity was observed. Laboratory trials indicated that F. coccophilum host range was restricted to the family Diaspididae, where mycosis was observed within the genera Hemeroblesia, Aspidiotus, Quadraspidiotus and Aonidiella. 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

Virus 5

Thursday, 8:00-9:45

Immune-gene responses in Penaeus monodon shrimp following infection with Gill-associated virus

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Over the past 30-40 years the production of cultured shrimp has increased exponentially to rival the volume of wild-cought 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 Roviriviridae family, whereas WSSV has a ~ 300 kb dsDNA genome and belongs to the Nodaviridae 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.

Subcellular localization and functional characterization of MdBV IkB - like protein N5 in Drosophila mnn2 cells

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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 factor components are targets 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-κB transcription factors. We found that MdBV Ank- H4 and -N5 proteins directly bind insect NF-κB. 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.

Studies of the subgenomic RNA3 and protein B2 of Wuhan Nodavirus

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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 8:30

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Studies of the subgenomic RNA3 and protein B2 of Wuhan Nodavirus

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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 8:00

147 STU

Immune-gene responses in Penaeus monodon shrimp following infection with Gill-associated virus

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Over the past 30-40 years the production of cultured shrimp has increased exponentially to rival the volume of wild-cought 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 Roviriviridae family, whereas WSSV has a ~ 300 kb dsDNA genome and belongs to the Nodaviridae 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 Papers

Virus 5

Thursday, 8:00-9:45
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 penetrate 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 (*AcMNPV*). To determine the role of gloverin during *AcMNPV* 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 *AcMNPV* budded virus (BV) in purified pro- or mature gloverin and compared 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**

**The impact of Dicer-2 and Wolbachia on antiviral protection in Drosophila**

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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- *Dizostrotoviridae*) 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 flies 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 9:30**

**Understanding the roles of p53 and the DNA damage response in baculovirus replication and baculovirus-induced apoptosis**

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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 sghydril oxide protein p53 (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 (Spf53) and Bombyx mori. Spf53 interacted with Ac92 by co-immunoprecipitation, similar to human p53, and over-expression of Spf53 induced apoptosis in Sf9 cells. Levels of endogenous Spf53 protein increased dramatically after treatment with DNA damaging agents or AcMNPV infection, and this increase in Spf53 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 Spf53 expression by RNAi (as verified by immunoblot) had no apparent effect on apoptosis induced by infection with a p53 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

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

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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 Cancer maenas; one estuarine species, Eriocheir sinensis and two freshwater species, Astrotomobius pallipes and Pacifastacus leniusculus. Exposure trials based upon natural (feeding) and artificial (intramuscular 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 STU

Behavior influences viral disease dynamics in the Caribbean spiny lobster

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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)

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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 may currently be 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 STU

In vitro cultivation of Hematodinium sp. and characterization of developmental stages

Peter H. Gaudet1; Richard Cawthorn1,2; Spencer Greenwood1,3; Dorota Wadowska1; Glenda Wright1

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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 discoloration) 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
Contributed Paper, Thursday 9:15 159 STU
Trichocyst development and their potential role in Hematodinium sp. survival
Peter H. Gaudet1,2; Richard Cawthorn1,2; Spencer Greenwood1,3; Dorota Wadowska1; Glenda Wright4
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Hematodinium sp. is a parasite 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
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Copepods are the natural food for many marine fish larvae, and the use of cultured copepods as live 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 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 multineurate 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 9:45 161 STU
Comparison of expression strategy of two birevidensoviruses, Peneaus stylirostris densovirus and Aedes albopictus densovirus
Hanh T. Phan1,2, François-Xavie Joussen3; Hiroko Shike1,4, Jane C. Burns4, Max Bergoin4, and Peter Tijssen1,2
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Based on similar size, organization of coding sequences, and structure of 5’- and 3’-termini of their genome, the Peneaus stylirostris densovirus (PstDNV) from shrimps and the Aedes albopictus (AalDNV), densovirus from mosquitoes are tentatively classified into the Birevidensovirus 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 robustly 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 birevidensoviruses 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.
Pioneer Women in Invertebrate Pathology and their Influence on the Field
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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

Contributed Paper, Thursday 13:30
Effects of additives and UV protectants on Plutella xylostella granulovirus efficacy to control Diamondback Moth (Plutella xylostella Linnaeus).


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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 (PxGV) was effective for suppressing 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 PxGV 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 PxGV suspension significantly improved the residual activity of PxGV after exposure to UV radiation. PxGV + Tinopal, PxGV + molasses, PxGV + lignin and PxGV + 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 LC₅₀ calculated for virus + molasses (5.2 x 10⁶ 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.

Commercial biopesticides based upon Helicoverpa armigera nucleopolyhedrovirus (HearNPV) 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 HearNPV is reduced. The present study compared the efficacy of HearNPV 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 HearNPV OBs. We are also studying similar effects on other legumes such as cowpea (Vigna unguiculata) and pigeonpea (Cajanus cajanus).

Control of Oriental Fruit Moth and Codling Moth with a new granulovirus isolate

Markus Züger1; Iris Kraaz2; Daniel Zingg3; Martin Andermatt4; Edith Ladurner5; Massimo Benuzzi6; Marketa Broklova7; Gyula Laszló8

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In addition to its key role as a pest on stone fruit, oriental fruit moth (Grapholita molesta) frequently migrates to pome 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 1.1x10⁶ OB/g diet in laboratory bioassays on codling moth larvae, ABC-V22 performed equally to the well known and highly effective Cydia pomonella Granulovirus (CrGV). Furthermore, the activity of this new isolate reaching a LC50 of 2.9x10⁶ 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%
Purification of an active fragment of Cry1Ie toxin from Bacillus thuringiensis

Shuyuan Gao1; Chunlu Zhang1; Xiaoyin Lin1; Yanrui Zhang1; Kanglai He1; Fuping Song2; Jie Zhang1
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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.

Spore-free insecticidal formulations based on cleaved Bacillus thuringiensis delta-endotoxins

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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 microflora condition of mammals was assessed. The ecological risks were evaluated as well.

Tenebrio molitor cadherin fragment, a potential additive in Bacillus thuringiensis Cry3Aa against vegetable Coleopteran larvae

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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.
Symposium Paper, Thursday 13:30

**Viruses of Forest Insect Pests In Honour of Basil Arif**

Organizers: Peter Krell and Kelli Hoover

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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/Tenthredine européenne de l’épinette (Glipinia heryciniae) introduced to North America in the 1930s. Both of these insects cause huge economic impacts (loss of 40 million m³ 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

**Baculoviruses of forest pests – their application from an European perspective**

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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* nucleopolyhedrovirus (LdMNPV) [Alphabaculovirus], saw flies by using *Neodiprion sp.* nucleopolyhedroviruses (NeleNPV, NeseNPV) [Deltabaculovirus], and the spruce budworm applying *Choristoneura fumiferana* granulovirus (ChfuGV) [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.
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, *Bassoeula fuscá* to Cry1Ab in South Africa, and *Diatroica virgífera virgífera* to Cry3Bb in the midwestern US. For Bt cotton, reported cases of resistance include *Helicoverpa zeá* to Cry1Aa and Cry2Aab in the southeastern US, *Pectinophora gossypiíla* to Cry1Aa in India, and *Helicoverpa armígera* 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 vegetable 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.

In Australia, monitoring *Helicoverpa* spp. 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. armígera* and *H. punctígéra*. 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. punctígéra*. 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.
Contributed Paper, Thursday 16:00

178 Persistence, stability and co-occurrence of Knellalenahizia solenopsae, S. invicta virus 1 (SINV-1), and SIN-V2 infections across years in Louisiana

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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 microsporidium Knellalenahizia 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)

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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 Tabulinoforma hippocastani on wasp development (30-day trials) and host choice. Uninfected D. coccinellae were provided uninfected and T. hippocastani-infected host beetles under laboratory conditions. All wasp progeny that developed within (and emerged from) T. hippocastani-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 or 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 (χ² = 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. hippocastani 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, Diaitreya saccharalis, and its parasitoid Cotesia flavipes

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Diaitreya saccharalis (Lepidoptera: Crambidae) is the major pest of sugarcane in Brazil. This pest is controlled by inadventive 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 LD₅₀ were 5.6 and 1230 spores for 1st and 3rd caterpillar instars, respectively. The LT₉₀ ranged from 42 to 9 days when treated with concentration from 10⁷ to 10⁸ spores/1st instar caterpillar. For the 3th instar, LT₉₀ ranged from 49 to 32 days at concentrations from 10⁷ to 10⁸ 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 natural species and revealed that Nosema sp. was pathogenic to Anticarsia gemmatalis, Pseudoplasmodia includens and Spodoptera albul a causing higher infection levels in the first two species. Heliotis virescens, 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

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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 dead parasitized 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. lymnaeae and progress of infection in L. dispar. Vegetative stages and primary spores of N. lymnaeae 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 1.0x10^9 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. lymnaeae.

Contributed Paper, Thursday 17:00

Evaluating methods of disinfecting Nosema ceranae-contaminated comb

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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 x 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.