

cymene,  $\alpha$ -Terpinolen,  $\beta$ -Phellandrene, Caryophyllene and  $\alpha$ -Caryophyllene. When aphids (*M. persicae*) were allowed to colonize these plants, VOC profiles again differed with regard to specific compounds and the amount produced. However aphids did not discriminate between tomato plants inoculated with different endophyte isolates compared to control plants. We speculate that the VOC pattern found may play a role for attraction of natural enemies (parasitoids), competing with the EPF for the herbivores.

CONTRIBUTED PAPERS Wednesday, 14:00-16:00

## MICROSPORIDIA 1

Contributed paper. Wednesday, 14:00 **169**

### Effects of the microsporidium *Nosema adaliae* on the multicoloured Asian lady beetle, *Harmonia axyridis*

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Originally imported for use as a biological control agent for pest insects, the multicoloured Asian lady beetle, *Harmonia axyridis* Pallas has itself become a pest in many areas of the world. While it is very effective for biological control, *H. axyridis* tends to displace many native lady beetle species and the alkaloids produced by these beetles may affect the palatability of wine and have adverse effects on human health. The geographic distribution of *H. axyridis* extends throughout North America into Nova Scotia and overlaps with the range of the native two-spotted lady beetle, *Adalia bipunctata* L. The microsporidium *Nosema adaliae* was recently found in a native population of *A. bipunctata* from Nova Scotia and the geographic overlap of *A. bipunctata* with *H. axyridis* provides the opportunity for this microsporidium to be transmitted horizontally to *H. axyridis* in nature. In this study, *H. axyridis* larvae were provided with a combination of uninfected and *N. adaliae*-infected eggs. All of the *H. axyridis* larvae that consumed *N. adaliae*-infected eggs became infected with the pathogen. *H. axyridis* larval development was prolonged significantly, depending on the number of eggs eaten. These results suggest that there is potential for *N. adaliae* to be transmitted to *H. axyridis* in nature if the larvae consume a sufficient number of microsporidia-infected eggs.)

Contributed paper. Wednesday, 14:15 **170-STU**

### Effects of two microsporidia from lady beetles on the green lacewing, *Chrysoperla carnea*

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The larvae of green lacewings, *Chrysoperla carnea* (Stephens), are generalist predators that feed on insect eggs, small caterpillars and other, soft-bodied insects. Lacewing larvae are commercially available for aphid control on various agricultural crops. It is common to use several types of biological control agents for controlling aphids at a given time to optimize pest control. Two-spotted lady beetles, *Adalia bipunctata* L., and convergent lady beetles, *Hippodamia convergens* Guerin-Meneville, are often released for aphid control in the same areas that lacewings are used. Two microsporidian pathogens infect these lady beetle species. Because lady beetles and green lacewings are often used

simultaneously for aphid control, it is possible for lacewing larvae to become infected with microsporidia when infected eggs are eaten. The main objective of this study is to determine if microsporidia from lady beetles (*T. hippodamiae* and *N. adaliae*) are transmitted to green lacewings and to examine the effects of these pathogens on lacewing larvae and adults.

Contributed paper. Wednesday, 14:30 **171**

### Features of the genomes of microsporidia in mosquitoes: status and preliminary findings

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The status and preliminary findings for full genome sequencing of two distantly related species of microsporidia with mosquitoes as type hosts will be presented. *Vavraia culicis*, the type species of the genus *Vavraia*, was originally described from *Culex pipiens*. Type material was not available and therefore *Vavraia culicis floridensis* isolated from *Aedes albopictus* in Florida was used for sequencing. *V. culicis* has a broad mosquito host range, is infectious for several species of Lepidoptera and characterized by having only uninucleate stages and produces uninucleate spores in multispore sporophorous vesicles. *Edhazardia aedis* is the type species for the genus and has a limited host range in mosquitoes but can only complete its life cycle in *Ae. aegypti*. *E. aedis* is polymorphic, producing 4 distinctive spore types. It is transmitted both horizontally and vertically and requires 2 generations of the mosquito host to complete the life cycle. Genome and transcriptome sequencing for *E. aedis* and *V. culicis floridensis* is completed. *V. culicis floridensis* has a genome size of approximately 6.1Mb while *E. aedis* is nearly an order of magnitude larger at approximately 51.3Mb, yet the gene content difference is smaller, with 2,773 and 4,190 predicted genes in *V. culicis* and *E. aedis* respectively. RNA-seq data has been analyzed for multiple time points in the life cycle of each species to validate predicted gene structures and to examine gene expression. Preliminary analysis of genome evolution and differential gene expression between life cycle stages will be presented.

Contributed paper. Wednesday, 14:45 **172**

### Multi-gene phylogeny applied to the taxonomy of microsporidian parasites of crustacean hosts

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*Hepatospora*, a recently erected genus, infects epithelial cells of the hepatopancreas of decapod crustaceans. We isolated *Hepatospora* sp. from three different crustacean hosts, inhabiting different habitats and niches; marine edible crab (*Cancer pagurus*), estuarine and freshwater Chinese mitten crab (*Eriocheir sinensis*), and the marine mussel symbiont pea crab, (*Pinnotheres piscum*). Isolates were initially compared using histology and electron microscopy revealing variation in size, polar filament arrangement and nuclear development.

Despite these morphological differences, sequence analysis of the partial SSU rDNA gene did not provide the resolution for distinguishing the isolates (>99% similarity). To investigate relationships between isolates, purified spore samples from the parasite infecting *E. sinensis*, and *C. pagurus*, were prepared for Illumina sequencing. Six additional gene sequences were mined from the resulting genomic data (RNA polymerase, Arginyl tRNA synthetase, Prolyl tRNA synthetase, Chitin synthase, Beta Tubulin and Heat Shock Protein 70). Primers were designed based on the above gene sequences to compare isolates, and to assess corresponding sequences in the genome of the isolate infecting pea crabs. Concatenated phylogenies using sequence data from the six genes revealed that *Hepatospora* isolates from the three different crustacean hosts are likely to be a single species. As such, the concatenated phylogeny supported that derived from analysis of SSU rDNA. Given the host, ecological and morphological distinction of the parasites infecting these three crabs, we provide further support for the concept that morphology is an inappropriate discriminator for even closely related taxa within the phylum, *Hepatospora* may form a widely distributed parasitic 'cline' within the hepatopancreas of aquatic crustacean hosts.

Contributed paper. Wednesday, 15:00 **173-STU**

#### Understanding the evolutionary loss of glycolysis in intranuclear crab microsporidians

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*Enterocytozoon bieneusi* is responsible for most cases of microsporidiosis in humans. Interestingly, an intranuclear microsporidian recently isolated from edible crabs appears to be a close relative of this human parasite on SSU based phylogenetic trees. Since microsporidians are known to be devoid of functional mitochondria and rely solely on glycolysis and energy import from their hosts, it was interesting to find that genes coding for glycolytic enzymes were absent from the genome of both parasites. Also, more recent genomic analyses of *Hepatospora* spp., another crustacean parasite show a similar loss of glycolytic enzymes alluding to a single loss of glycolytic capabilities prior to the divergence of the Enterocytozoonidae lineage.

Absence of glycolysis may be compensated by the increase in host-ATP availability created by the aggregation of host mitochondria around microsporidian meronts, a feature often observed in microsporidian infections. However, *E. cankeri* is an intranuclear parasite and hence, physically walled off from the host mitochondria. This may highlight the presence of a novel host nuclei-dependent metabolic process. To this end, phylogenetic and structural domain analyses on the first enzyme of the glycolytic pathway, hexokinase has revealed that it is severely mutated in deep branching microsporidia hinting to a different substrate-specificity adaptation. Most intriguing is the adjunction of a PTPA domain on the hexokinase of *E. cankeri*. This is the first time severe mutations of hexokinase conserved domains have been documented in eukaryotic cells and our current efforts are directed towards understanding whether these mutations are associated with loss of enzyme function.

Contributed paper. Wednesday, 15:15 **174-STU**

#### Temporal trends and the effect of seasonal temperature on the prevalence of *Nosema* spp. in *Apis mellifera* in north-east Germany

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*Apis mellifera* (European honey bee) has long been host to *Nosema apis* and *Apis ceranae* (Asian honey bee) to *Nosema ceranae*. Natural infections of *N. ceranae* were first discovered in *A. mellifera* colonies in 2005. *N. ceranae* has already replaced *N. apis* in the warmer European countries and, as an emergent pathogen, is very competitive. It has been found to be more better adapted to higher temperatures than *N. apis* and is considered more virulent than *N. apis*. Lab experiments have shown the differential effect of temperature on *N. apis* and *N. ceranae* with respect to spore germination and virulence of the pathogen within the host.

Our study is based on a 10-year (2005-2014) cohort study of roughly 20 apiaries from north-east Germany, monitored in autumn and spring. Trend analyses show a significant increase in the prevalence of *N. ceranae* and a decrease in that of *N. apis*, suggesting the gradual replacement of *N. apis* by *N. ceranae*. Weather aggregates from different periods of the season preceding the colony sampling were tested against the proportion of infected colonies. They reveal considerable variability in their effects on *Nosema* prevalence. We are able to confirm, for the first time, the effect of seasonal temperatures on the prevalence of *Nosema* in honey bee colonies. Effect of North Atlantic Oscillation (NAO) indices were also tested as proxies for seasonal weather and were found to be reasonably good predictors of *Nosema* prevalence.

Contributed paper. Wednesday, 15:30 **175-STU**

#### Characterising putative virulence factors of the bee pathogen *Nosema ceranae*

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Microsporidia are obligate intracellular eukaryotic parasites related to fungi, possessing greatly reduced genomic and cellular components. The microsporidian *Nosema ceranae* threatens the two economically important pollinators, honey (*Apis mellifera*) and bumble (*Bombus* species) bees and has been causally linked to colony collapse disorder. Nosemosis has a complex epidemiology affected by host, pathogen and environmental factors. Although a draft of the *N. ceranae* genome has been published, the molecular basis underpinning pathogenicity is not known. The lack of established culturing techniques and a tractable genetic system necessitates use of model systems for both host and parasite such as *Saccharomyces cerevisiae*. We hypothesise effectors essential to disease progression exist amongst *N. ceranae* secretome genes. In this study we have started characterising these genes using Gateway® cloning technology and identify candidate effectors by their expression in *S. cerevisiae*. We offer experimental data supporting the identities of NcORF-01664 and NcORF-01663 as polar tube proteins (PTP) 1 and 2

respectively and identify a putative PTP4 through their capacity to induce morphological deformities in *S. cerevisiae*. We also show two unknown proteins are targeted to lipid droplets which could function to mobilise resources from this energy-rich organelle. In the future we hope to confirm this function is retained in a system more closely related to the insect host tissue using the *D. melanogaster* Gal4/UAS method. Increased knowledge on virulence factors and disease progression will ultimately lead to disease mitigation.

Contributed paper. Wednesday, 15:45 **176**

**Detection of Microsporidia in Gammarids in the Delta of the Kuban River (Azov Sea, Russia)**

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Gammarids of the Kuban river basin were surveyed for microsporidia infections at two sites: a) Azov sea coast close to the river Protoka mouth and b) a quarry lake 11 km eastwards from the sea. At the first site, a population of *Dikerogammarus villosus* was abundant in the intertidal zone. In June, 5 out of 100 specimens displayed developed infections with a monomorphic microsporidium. Its ribosomal RNA gene sequence showed high (above 99%) similarity to *Anncalia algerae* with no variability between isolates from individual hosts. In July, the microsporidia were absent in gammarids (N=100). At the second site, in the quarry lake and neighboring ditches, there was an abundant population of *Gammarus* sp. infected with a dimorphic microsporidium at the rates of 100% in May and 50-80% in June. Sequencing of four cloned SSU rRNA gene amplicons (ca 900 bp long) from an individual host sample produced four distinct (97.8-99.4% similarity) haplotypes, suggesting infection with multiple genetically distinct isolates or species of genus *Dyctiozoela*. The latter taxon unites common and widespread gammarid-infecting microsporidia and revealing a new species of *Dyctiozoela* in these hosts is quite expected. Conversely, the detection of an *A. algerae*-like parasite in gammarids is somewhat unusual, though logical given the broad host range of *A. algerae* and its ability to develop in amphipods upon injection of spores into the hemocoel. This pathogen has potential risk for human infection and should be taken into account when considering safety of public beaches. Supported by RFBR, 13-04-00284 and 14-04-91176.

CONTRIBUTED PAPERS Wednesday, 14:15-15:45

**MICROBIAL CONTROL 3**

Contributed paper. Wednesday, 14:15 **178-STU**

**Synthesis and Characterization of fungus mediated silver nanoparticle for the toxicity on filarial Vector,**

***Culex quinquefasciatus***

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Larvicidal activities on myco-synthesized silver nano-particles (AgNPs) against filarial vector, *Cx. quinquefasciatus*. The AgNPs synthesized by filamentous fungus, *Penicillium verrucosum*. Characterized by UV-Vis spectrophotometer, Fourier transform infrared spectroscopy, scanning electron microscopy, and transmission electron microscopy. Furthermore, laboratory evaluation of fungus mediated silver nano-particle against larvae and pupae of *Cx. quinquefasciatus*. The characterization studies confirmed the spherical shape and size (3–24 nm) of silver nano-particles. The efficacy of fungus AgNPs tested concentrations of 25 and 50 ppm against L1, L2, L3 and L4 instar larvae of *Cx. quinquefasciatus*. The LC<sub>50</sub> (LC<sub>90</sub>) values are 4.91 (8.13), 5.16 (8.44), 5.95 (7.76) and 7.83 (12.63) in L1 to L4 instar at 25 ppm. Whereas, LC<sub>50</sub> (LC<sub>90</sub>) were 5.24 (8.66), 5.56 (8.85), 6.20 (10.01) and 7.04 (10.92) in L1 to L4 instars treated at 50 ppm. The mortality rates were positively correlated with the concentration of AgNPs. Significant (P<0.05) changes in the larval mortality was also recorded between the period of exposure against all instar of larvae of *Cx. quinquefasciatus*. These finding use of fungus synthesize silver nano-particles is a rapid, eco-friendly, and a single-step approach and potential mosquito larvicidal agents.

Contributed paper. Wednesday, 14:30 **179-STU**

**Entomopathogenic fungi as endophytes: interaction with phytohormones**

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With increasing interest in entomopathogenic fungi as endophytes (EPPF) in biological control strategies, there is a need for more background information on the interaction of these fungi with their host plant metabolism. Several studies have already reported on changes in the dry weight of plants when endophytically colonized by these EPPFs; however, a more detailed understanding of plant-fungus-interactions is missing. We measured phytohormone levels in plants with regard to the hypotheses that i) EPPFs produce phytohormones as fungal secondary metabolites when growing within plant tissues or ii) that plants react to the presence of EPPFs by increasing/decreasing their phytohormone production. We inoculated the seeds of tomato (*Solanum lycopersicum*) and cotton (*Gossypium hirsutum*) plants with one strain of *Beauveria bassiana* and three different strains of *Metarhizium anisopliae*, and grew these plants under standardized conditions in the greenhouse. We used LC-MS to analyse several phytohormones (including Salicylic Acid (SA), Abscisic Acid (ABA), Indolic Acetic Acid (IAA), Salicylic Acid Glucoside (SAG), and Jasmonic Acid (JA)) in eight weeks old leaves of these plants. The results will be discussed with regard to induced plant responses as well with regard to potential influences on herbivore-plant-interactions.

Contributed paper. Wednesday, 14:45 **180**

**Pathogenicity of three entomopathogenic fungi on larvae and adults of the sisal weevil: The less the better?**

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