

BV isolates using four core genes (*polh*, *lef-8*, *lef-9*, *pif-2*) from which we delimited virus species, to obtain a comprehensive timed molecular BV species phylogeny. We then used a combination of phylogenetic (BV and insects) and ecological (host range of BVs, host plant range of lepidopteran hosts) data to address the following hypothesis: BVs are host specialists and show high levels of phylogenetic conservatism, BVs have the same ages as their lepidopteran hosts and the host plants of the insects drive also the evolution of BVs. We found that in general, hosts primarily induced BV species speciation over a short timeframe. But on a larger evolutionary scale, the insect-host co-evolutionary relationship signal is confused. Surprisingly we revealed that insect host plant specificity contributed largely to BV evolutionary history.

CONTRIBUTED PAPERS Wednesday, 10:30-12:15

## FUNGI 5

Contributed paper. Wednesday, 10:30 **162**

**An entomopathogenic strain of *Beauveria bassiana* against *Frankliniella occidentalis* with no detrimental effect on the predatory mite *Neoseiulus barkeri***

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Among 28 isolates of *Beauveria bassiana* tested for virulence against *F. occidentalis* in laboratory bioassays, we found strain SZ-26 as the most potent, causing 96% mortality in adults at  $1 \times 10^7$  mL<sup>-1</sup> conidia after 4 days. The effect of the strain SZ-26 on survival, longevity and fecundity of the predatory mite *Neoseiulus (Amblyseius) barkeri* Hughes were studied under laboratory conditions. The bioassay results showed that the corrected mortalities were less than 4 and 8% at 10 days following inoculation of the adult and the larvae of the predator, respectively, with  $1 \times 10^7$  conidia mL<sup>-1</sup> of SZ-26. Furthermore, no fungal hyphae were found in dead predators. The oviposition and postoviposition durations, longevity, and fecundity displayed no significant differences after inoculation with SZ-26 using first-instar larvae of *F. occidentalis* as prey in comparison with untreated predator. In contrast, the preoviposition durations were significantly longer. Observations with a scanning electron microscope, revealed that many conidia were attached to the cuticles of *F. occidentalis* at 2 h after treatment with germ tubes oriented toward cuticle at 24 h, penetration of the insect cuticle at 36 h, and finally, fungal colonization of the whole insect body at 60 h. In contrast, we never observed penetration of the predator's cuticle and conidia were shed gradually from the body, further demonstrating that *B. bassiana* strain SZ-26 show high toxicity against *F. occidentalis* but no pathogenicity to predatory mite.

Contributed paper. Wednesday, 10:45 **163-STU**

**Interactions between the insect pathogenic fungus *Metarhizium*, the wheat pathogen *Fusarium culmorum* and the mycoparasitic fungus *Clonostachys rosea***

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The current study was conducted to determine if wheat seeds co-inoculated with the insect-pathogenic fungus *Metarhizium* (three species) and the mycoparasitic fungus *Clonostachys rosea* are protected from both insect pests and plant pathogens. The experiment was done in two parts: First, a co-infection bioassay was performed to determine if the virulence of *Metarhizium* was affected by the presence of other fungi by co-treating *Tenebrio molitor* larvae with combinations of *Metarhizium*, *C. rosea*, and the wheat pathogen *Fusarium culmorum*. Second, wheat seeds were co-inoculated with the both beneficial fungi and compared to single inoculations of the effects on *F. culmorum* when allowed to grow for two weeks under controlled laboratory conditions. The resulting root systems were then placed with *T. molitor* larvae which were evaluated daily for mortality. Pathogenicity to insect persisted in all treatments, but *Metarhizium* virulence was affected by co-treatments with other fungi. Root-infection by *F. culmorum* was not reduced directly by the presence of *Metarhizium* while *C. rosea* reduced *F. culmorum* infection and this effect was not diminished in combination with *Metarhizium*. The results of this study suggest that combination of beneficial fungi may effectively protect roots from both pathogens and insects pests..

Contributed paper. Wednesday, 11:00 **164**

**Diversity, ecology and virulence of entomopathogenic fungi isolates naturally infecting the red palm weevil *Rhynchophorus ferrugineus* (Olivier) in the Mediterranean Basin**

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The red palm weevil, *Rhynchophorus ferrugineus* (Olivier) (Coleoptera: Curculionidae), and the moth *Paysandisia archon* (Burmeister) (Lepidoptera: Castniidae) are considered nowadays the most important palm pest worldwide. Current tactics commonly used to manage the weevil are based on chemical control, although the use of these compounds is hampered by several environmental concerns. In recent years, the *R. ferrugineus* (Rf) microbial control potential of entomopathogenic fungi (EPF) has been highlighted. In this work, several strains of EPF have been isolated from diverse naturally infected specimens of both species, found in different countries through the Mediterranean Basin. Firstly, the usefulness of the elongation factor 1-alpha (EF1-α) region, the nuclear intergenic region BLOC and inter simple sequence repeat (ISSR) or microsatellite markers were assessed as *R. ferrugineus* EPFs diagnostic tool, alone or in combination, and relationships among the Mediterranean *Beauveria* and *Metarhizium* isolates obtained from the red palm weevil were inferred.

Secondly, the effect of diverse environmental parameters such as temperature, humidity and UV-B radiation were assessed on germination and colony growth of these EPFs strains as function of their genealogy and geographic origin. Finally, virulence of selected isolates was tested against both Rf larvae and adults.

Our results show a distribution pattern of *Beauveria bassiana* through the Mediterranean Basin, possibly associated with the host insect dispersion, with the same genetic group presented throughout the European distribution area of phytophagous. Furthermore, several differences were observed between the different genetic groups found, regarding the different factors analyzed: temperature, humidity, UV-B radiation and virulence.

Contributed paper. Wednesday, 11:15 **165\_STU**

**Recovery and detection of an entomopathogenic endophyte: overcoming the challenges involved**

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The prospect of utilising entomopathogenic fungi, such as *Beauveria* spp., as endophytes to enhance their biological control activity is presently a highly topical area of research. However, the endophyte-host relationship is complex and the associated methodology for defining and recovering endophytic strains wrought with problems. A review of the literature on endophyte isolation methods indicated the need for developing a methodology for reliable molecular detection of *Beauveria* spp. *in planta*. The method that was developed included a stringent surface sodium hypochlorite and ethanol based sterilisation with the protocol optimized specifically for propagules of *Beauveria* spp.. This was followed by treatment of plant tissue with propidium monoazide (PMA<sup>TM</sup>) to exclude surface DNA contamination from subsequent PCR. A nested PCR/RT-qPCR protocol capable of detecting as little as 32 fg was developed using novel primer sets designed from the translation elongation factor 1- $\alpha$  gene (TEF). Additionally, epiphytic DNA was isolated separately using a benzyl chloride treatment in order to determine any corresponding occurrence of *Beauveria* with endophyte positive samples. Freshly inoculated samples of *Zea mays* and *Solanum lycopersicum* were surface sterilized using the optimized method and various controls were included for comparison to determine at which stage(s) *B. bassiana* remained viable. Results suggest that *Beauveria* DNA contamination and viability after surface sterilisation is a common and confounding issue associated with endophyte detection and isolation. However, this may be overcome with the improved methodology described here, which delivers reliable detection of endophytic strains.

Contributed paper. Wednesday, 11:30 **166-STU**

**Intense spatio temporal pattern in pathogen-host interaction between *Pandora formicae* and *Formica rufa***

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*Pandora formicae* (Entomophthorales, Entomophthoromycota) is a pathogen of the common red wood ant (*Formica rufa*), causing symptoms of "summit disease", where ants attacked by the fungus place themselves on an elevated position before death and sporulation, enabling the pathogen to release infectious spores widely. This facilitates fungal transmission but puts the ant colonies at an enhanced risk of a lethal disease outbreak. Ant workers, on the other hand, respond by removing the cadavers from the nest surroundings, by that lowering the load of conidia, but at the same time putting them at risk while protecting the colony from this hazard. Detailed mapping of the cadavers around an ant nest, twice a day for three subsequent days, three times during one season, shows how ants' behavioral response keeps the fungus prevalence 'at hold'. It also shows the uniqueness of this interaction, the only known example of an entomophthoralean fungus infecting a social insect host, and an evolutionary adjustment of fungal

life strategy to maintain itself in the host population without causing rapid epidemics.

Contributed paper. Wednesday, 11:45 **167**

**Patterns of host adaptation in fly infecting *Entomophthora* species**

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Insect pathogenic fungi (IPF) differ widely in their capability to infect different hosts. Some are generalists and will, given a sufficient number of infectious spores are present, infect almost any species of insect (e.g. Hypocrealean *Metarhizium* and *Beauveria*). Members of a different main IPF phylum Entomophthoromycota generally have more narrow host-ranges where some species for example only infect aphids or only locusts. Certain species (or strains) are even more host specific and are only known to infect a single or very few taxonomically related insect species under natural conditions (e.g. *Entomophthora*, *Strongwellsea* and *Entomophaga*). Species diversification of the obligate IPF within Entomophthoromycota thus seems to be primarily driven by co-evolutionary host adaptation to specific insect families, genera or species-complexes, but the underlying genetic factors of host adaptation in this fungal order are largely unknown and leave many unanswered questions. For example are the numbers of virulence factors increasing, or decreasing when fungal pathogens adapt to a narrow range of potential hosts? And, are host specialization based on many genetic changes with small effect or few with large effect? Here we examine closely related species within the *Entomophthora muscae* species complex: *E. muscae* s. str. infecting the common housefly *Musca domestica* and *E. muscae* s.l. strains infecting the cabbage fly *Delia radicum*. We use RNA-seq based comparative transcriptomics to unravel genetic differences and similarities in order to detect patterns of host-specific molecular adaptation.

Contributed paper. Wednesday, 12:00 **168-STU**

**Plant volatile organic compound manipulation by endophytic entomopathogenic fungi**

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The potential of entomopathogenic fungi (EPF) to live as endophytes in various plant tissues has been demonstrated several times in recent years. However, the effects of plant colonization by these endophytes on the metabolism of the colonized plants have been only rarely addressed. We analyzed the volatile organic compound (VOC) profiles of tomato plants (*Solanum lycopersicon*) inoculated with three strains of *Beauveria bassiana* and a plant pathogen biocontrol agent (*Trichoderma koningiopsis*) compared to control plants. We hypothesized that EPF colonized plants should be more attractive for herbivores, mediated by the VOC profiles, but should not exhibit differences when colonized by the plant pathogen antagonist. We found that *B. bassiana* and *T. koningiopsis* inoculated plants had significantly modified VOC profiles, with marked differences between different isolates. Some of the compounds up- or down-regulated are known to play a role in plant-herbivore interactions such as  $\alpha$ -pinene,  $\beta$ -

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.