

Poster / Dis. Ben. Invertebr. Wednesday, 16:30. **DB-11**

What Kind of Insects Do You Like?

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Insect constitute the largest and most diverse group of animals on world and also serve as the hosts or nutrient sources. In addition, several insects have a strong influence on people's emotion. To utilize the preference and interest of insects in the field of mental healthcare, a survey study was conducted with individual living in Korea. As results, the most people had a high preference and interest of insect, but some were disagreeable to the insect itself. The preference and interest of insect were high on male, adult and practitioner experienced insect-related events than female, student and non-practitioner, respectively. The most favored insects were familiar or pet insects such as *Papilio xuthus*, *Lucanus maculifemoratus*, *Allomyrina dichotoma* and Lampyridae. These results may be useful to develop a healing program for mental healthcare using insects. Further research is needed to determine the effects of these insect in the mental therapy for this purpose.

Poster / Dis. Ben. Invertebr. Wednesday, 16:30. **DB-12**

A muscle-infecting microsporidium infecting pink shrimp (*Pandalus montagui*) from Europe: closing in on the type species of *Thelohania*?

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The type species of the genus *Thelohania*, *T. giardi* was described infecting European brown shrimp (*Crangon crangon*) in the late 1800's. Although never rediscovered, recent work describing *T. butleri*, a similarly octosporous microsporidium infecting Canadian pink shrimp (*Pandalus jordani*), provided evidence that *Thelohania* (containing parasites of marine shrimp, freshwater crayfish, and ants) is polyphyletic and in need of significant revision. This work led to proposals that only marine forms should be considered as true members of the genus and that effort should be applied to rediscover the type species. In this study, we describe a novel microsporidium infecting another pandalid shrimp, *P. montagui* from Europe using histological, ultrastructural and phylogenetic data. Although the parasite does not display the characteristic morphological features of either *T. giardi* or *T. butleri* (8 spores contained within each sporophorous vesicle), phylogenetic analysis places it closest to *T. butleri* (91% similarity, 100% coverage of 937bp fragment of SSU rDNA gene) within the broader microsporidian tree. Previous work from our laboratory has focussed on the potential for morphological plasticity within Microsporidia infecting the musculature of marine crustaceans. To this end, we propose that despite divergence in form from the type species of *Thelohania*, the close phylogenetic relationship to *T. butleri* suggest that the parasite in *P. montagui* is a species of *Thelohania*. In addition, we provide further evidence that closely related taxa can display wide morphological variance and, that marine thelohanids may display a level of intra-generic plasticity which nullifies the use of morphology in their taxonomy.

FUNGI

Poster / Fungi. Wednesday, 16:30. **FU-1-STU**

Monitoring of entomopathogenic fungi in *Metarhizium* and *Beauveria* treated fields

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Application of entomopathogenic fungal strains for the control of cockchafer grubs was investigated in sour cherry orchards. Safety like possible effect of the inoculum on natural soil microbiota as well as efficacy and fate of these fungi need to be investigated. The applied fungal strains have wide host range, thus we have to determine the risks of their use during repeated long-term applications. Different inoculation methods were compared and the persistence of inoculum was monitored in the soil and on target and non-target organisms. One year after treatments we collected soil samples and grubs from un-treated and treated areas and re-isolated the fungi on selective media. Furthermore we applied PCR analysis for the identification of our *Metarhizium anisopliae* strains. According to Ya Li & Shuang Hu-Cai (2011) we used a species-specific primer for the detection of fungus. We were able to detect the presence of *Metarhizium* strains. Neither another entomopathogens (*Beauveria*, *Lecanicillium*), or other fungi like fusaria gave positive signal with the *Metarhizium*-specific primers. Furthermore, the presence of *M. anisopliae* was detected in about 10 percent of untreated soil samples. It proves that *Metarhizium anisopliae* can be found in the original soil mycobiota, although at a very low frequency. Research was supported by the grant **GOP-1.1.1-11-2012-0059** „Development of environment friendly product with the use of entomopathogenic organisms”.

Poster / Fungi. Wednesday, 16:30. **FU-2**

Distribution of insect-pathogenic soil fungi in agricultural and forest ecosystems in Georgia

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Entomopathogenic fungi naturally occurring in the soil represent a reservoir of antagonists to insect pest. Local strains of such fungi may be adapted to their environment and are of particular interest for usage in biological control. Georgia has a high diversity of altitudes, eco-systems and cropping system and may offer special opportunities for studies of insects pathogens. Soil samples were obtained in 2012-2013 from 8 different geographical sites at different altitudes (600-2200 m a.s.l.), representing different agricultural and forest ecosystems, National parks of Georgia. A total 161 soil samples representing 45 locations were analysed using the insect bait method (Waxworm, *Galleria mellonella* L. and Mealworm *Tenebrio molitor*). The following entomopathogenic fungal taxa were found: *Beauveria bassiana* s.l., *Beauveria brongniartii*, *Metarhizium* spp., *Lecanicillium* sp. *Isaria* sp. Also, we isolated *Aspergillus flavus*. The most abundant species was *Beauveria bassiana* (41,4%) and *Metarhizium* sp. (49,4%) from the total number of isolates. Three isolates of both *Metarhizium* and *Lecanicillium*

were found, while only one *Beauveria brongniartii*. Interestingly, no entomopathogenic fungi were isolated from six of the soil samples. In these locations, *B. bassiana* was predominantly recovered more often from soils of natural habitats, while *Metarhizium* spp. were recovered mostly in agricultural habitats. Our study included a limited number of samples, and more extended studies may reveal additional information about the occurrences of these fungi in different habitats and geographical zones of the South Caucasian region.

Poster / Fungi. Wednesday, 16:30. **FU-3**

Diversity of Entomopathogenic fungi in different citrus cropping systems in Brazil

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Biodiversity studies of entomopathogenic fungi (EF) in agroecosystems are very important to understanding the ecology of indigenous populations, their contributions to pest control and the impact of agricultural practices on their populations. The objective of this study was to investigate the natural occurrence of EF in citrus in São Paulo State, Brazil. Samples were collected in four fields with conventional cropping systems (Santa Barbara D'Oeste, Conchal, Nova Europa and Bebedouro), one organic field (Itirapina) and some abandoned fields in Itapetininga, Anhembi, Conchal, Corumbataí, Limeira and Araras during one year (2013-2014). The EF were isolated from soil samples by selective medium and the "Insect Bait" method using *Tenebrio molitor* larvae, and from pest samples by direct transfer onto PDA medium. The Hypocreales fungi isolated from soil by selective medium were *Metarhizium* sp. (18.9% of 174 soil samples) followed by *Beauveria* sp. (14.3%) and *Isaria fumosorosea* (8%). Using the "Insect Bait" method *Metarhizium* sp. was recovered from 75.9% of the soil samples and *Beauveria* sp. from 1.7% of samples. The insect pests found infected by EF were the citrus snow scale, *Unaspis citri* (Hemiptera: Diaspididae) infected with *Beauveria* sp. and *Pochonia* sp., the sharpshooters (Hemiptera: Cicadellidae) with *Beauveria* sp., the whitefly *Dialeurodes citri*, and citrus blackfly *Alurocanthus woglumi* (Hemiptera: Aleyrodidae) with *Aschersonia* sp., green scale *Coccus viridis* (Hemiptera: Coccidae) with *Lecanicillium* sp., and two unidentified Lepidopteran with *Cordyceps* sp. in organic and abandoned citrus fields. In the abandoned fields the density of EF in the soil was lower than the conventional and organic fields.

Poster / Fungi. Wednesday, 16:30. **FU-4**

The Entomopathogenic Fungus *Isaria* for Pest Insect Control in Vegetables

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The growing area of vegetables in the EU covers more than 3,000,000 ha. BIOCOTES is an EU funded project to provide fundamental information for the development of plant protection products, based on biocontrol agents (BCA). Currently, the common control of various insect pests is mainly by synthetic insecticides. Nevertheless, several pest insects cause considerable damage in agriculture due to resistance to pesticides.

The aim of the BIOCOTES work package is to develop a new

fungal BCA for pest insect control in open field crops and in greenhouses. Presently, we investigate the integration of entomopathogenic fungi into a control strategy. Within different treatments and pre- and post-harvest applications in protected and non-protected cropping systems, we compare the efficacy of at least 10 *Isaria* spp. strains under different laboratory conditions. Moreover, the host range of these strains will be screened, in order to determine the relationship of clade specific differences between virulence and pathogenicity factors. Additionally, the effect on beneficial insects like the predatory mite *Typhlodromus pyri* and the seven-spot ladybird, *Coccinella septempunctata*, will be evaluated to assess the possibility for implementation of entomopathogenic fungi in an integrated pest management strategy. As entomopathogenic fungi are known to produce a wide range of secondary metabolites as, e.g., antibiotics or repellents, selected strains will be screened for secondary metabolites and enzyme activities. Actually, first results will be presented.

Poster / Fungi. Wednesday, 16:30. **FU-5**

Prevalence of *Beauveria pseudobassiana* among tick-associated fungal isolates from the Republic of Moldova

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Human and animal disease transmitting hard ticks (Acari: Ixodidae) are of eminent concern for public health and animal farming. Alternatives to tick control by chemical acaricides are highly solicited, and one intensively evaluated biocontrol strategy is based on the use of tick-pathogenic filamentous fungi. An indispensable prerequisite of the development of tick-derived fungal isolates into registered myco-acaricides is their sound taxonomic characterization.

Using a molecular taxonomic approach based on phylogenetic reconstruction from both internal transcribed spacer (ITS) and protein-encoding gene sequences, a set of fungal strains isolated from ixodid ticks in the Republic of Moldova that had previously been assigned to the species *Beauveria bassiana*, together with further tick-derived fungal isolates from different geographic locations in Europe and the North America was characterized at the genus and species level. All fungi investigated were conclusively assigned to one of the two "hyphomycete" genera, *Beauveria* or *Isaria* (Ascomycota; Hypocreales; Cordycipitaceae). Within the genus *Isaria*, two species, *Isaria farinosa* and *Isaria fumosorosea*, were equally represented. Within the genus *Beauveria*, the species *Beauveria pseudobassiana* was found to strongly prevail among the isolates from Moldova. In particular, the previous classification as *B. bassiana* could not be confirmed for any of the correspondingly characterized tick-pathogens from Europe and North America. The data presented motivate the hypothesis that within the genus *Beauveria* specific adaptation to ticks might have occurred in the species *B. pseudobassiana*. However, to test this hypothesis, a more extensive molecular taxonomic survey carefully reconsidering previous taxonomic assignments of tick-derived fungal isolates is indispensable.

Poster / Fungi. Wednesday, 16:30. **FU-6**

Diversity and abundance of entomopathogenic fungi on strawberry crops in Brazil

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The aim of this study was to characterize the diversity and abundance of entomopathogenic fungi in arthropods on leaves of strawberry and of spontaneous herbaceous plants from the crop borders as well as from soil samples of organic and conventional fields in. The aboveground pests were collected from the crop and from the crop border vegetation at four localities of the Minas Gerais state in Brazil and were incubated in high moisture and inspected for fungi, daily. Two methods were used for isolation of entomopathogenic fungi from soil: selective media (SM) and insect baiting (IB) with *Tenebrio molitor*. No entomopathogenic fungi were observed in the aboveground insect pests, while eight mites were infected with *Neozygites floridana*. Pooling all soils samples revealed that *Metarhizium* spp was the most common fungus (73%-SM / 97.9%-IB), followed by *Beauveria* spp (22%-SM / 1.7%-IB) and *Isaria* spp. (5%-SM / 0.4%-IB). Diversity and abundance of entomopathogenic fungi was not much different between organic and conventional fields. For organic cropping alone the following fungi were isolated: *Metarhizium* spp (58.5%-SM / 97.5%-IB), *Beauveria* spp (34%-SM / 2.5%-IB) and *Isaria* spp. (7.5%-SM / 0%-IB) and for crop border vegetation in organic systems *Metarhizium* spp (82.4%-SM / 96.7%-IB), *Beauveria* spp (17.4%-SM / 2.6%-IB) and *Isaria* spp. (0.2%-SM / 0.7%-IB). For conventional cropping: *Metarhizium* spp (83.2%-SM / 98%-IB), *Beauveria* spp (16.8%-SM / 1%-IB) and *Isaria* spp. (0%-SM / 1%-IB), and for crop border vegetation around conventional crops: *Metarhizium* spp (86.1%-SM / 100%-IB), *Beauveria* spp (4.3%-SM / 0%-IB) and *Isaria* spp. (9.6%-SM / 0%-IB). The on-going studies on the intra-specific diversity will reveal the role of the crop borders as a reservoir of these generalist natural enemies.

Poster / Fungi. Wednesday, 16:30. **FU-7**

Abundance and diversity of *Metarhizium* spp. in an agricultural landscape in Sweden

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Entomopathogenic fungi belonging to the genus *Metarhizium* are important regulators of insect populations, including agricultural pests, and products based on these fungi have been applied in augmentation biological control of different pest insects. As sustainable agriculture and implementation of integrated pest management is gaining attention, the interest in establishing conservation biological control strategies is also growing. In conservation biological control, habitats or agricultural practices are adjusted to enhance the abundance of resident natural enemies, i.e. the biological control agent. Such approaches require a profound understanding of the control agent's life cycle and its ability to survive in different environments. However, abundance and diversity of these entomopathogenic fungi in Sweden have not been evaluated. In this study, we therefore investigate the occurrence of indigenous *Metarhizium* spp. in transects of a cereal field, a permanent grassland and an unmanaged forest site in Uppland, Sweden using cultivation-

dependent techniques as well as quantitative PCR. A collection of new *Metarhizium* isolates from the different habitats will be established, and strains will be characterized by PCR and genotyping. Factors such as soil management and vegetation will be evaluated for their effect on the abundance and diversity of *Metarhizium* spp. This study will generate new information on the potential of using *Metarhizium* for insect pest control in Sweden. Hence, it will facilitate the development of *Metarhizium* based biological control approaches including both augmentation as well as conservation biological control and the use of these approaches in sustainable farming systems in Sweden.

Poster / Fungi. Wednesday, 16:30. **FU-8**

Diversity and distribution of entomopathogenic fungi in Czech Republic soils

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A survey of entomopathogenic fungi was carried out in cultivated and uncultivated soil habitats in Czech Republic. A total of 189 soil samples were collected during October 2013. Two different methods of isolation were employed, selective media containing cicloheximide and SYLLIT 65 WP and *Tenebrio molitor* bait method. Entomopathogenic fungi were detected in all collected soil samples by using selective media, but not with the second isolation method. Eight different taxa belonging to five different genera were encountered by using morphological and molecular identification (ITS and EF 1- α molecular markers). The two more common taxa were unnamed species designated as *Lecanicillium* sp. (14%) and *Metarhizium anisopliae* (44.5%). Additionally, uncultivated soils showed a higher richness in entomopathogenic fungi than cultivated ones.

This is the first time that a monitoring study for the natural occurrence of entomopathogenic fungi was developed covering all Czech Republic. This study constitutes a valuable source for the discovery of indigenous isolates that can be applied in biological control strategies.

Poster / Fungi. Wednesday, 16:30. **FU-9**

Entomopathogenic fungi as plant growth enhancers

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Entomopathogenic fungi such as *Beauveria bassiana*, *Metarhizium brunneum*, and *Isaria fumosorosea* are primarily used for managing pests. A preliminary study showed that treating the roots of strawberry transplants with *B. bassiana* significantly promoted its growth compared to untreated plants or those treated with a commercial plant growth enhancer. In another study, soil treatment of strawberry plants with *M. brunneum* appeared to help plants withstand twospotted spider mite (*Tetranychus urticae*) infestations compared to untreated plants. These studies suggest that entomopathogenic fungi could be promoting plant health and growth through mycorrhizal interaction. A study was conducting by soil treatment of potted cabbage plants with various commercial products based on entomopathogenic fungi - *B. bassiana*, *M. brunneum*, *I. fumosorosea*, mycorrhizal fungus - *Rhizophagus irregularis*, and a formulation based on bacterial and fungal combination - *Azorhizobium caulinodans*, *Bacillus subtilis*, *Pseudomonas*

phaseoli, *Rhizobium phaseoli*, and *Trichoderma virens*. Impact of these treatments on plant development will be discussed. Preliminary data show superior growth of cabbage plants treated with *B. bassiana*.

Poster / Fungi. Wednesday, 16:30. **FU-10**

The entomopathogenic fungus *Beauveria bassiana* improves the growth of *Triticum aestivum* and *Triticum durum*

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The main role of Entomopathogenic Fungi (EF) is to kill insect. However, it was recently discovered that many EF, especially hypocrealean ascomycetes, have additional not fully understood ecological roles. This research deals with the effect that EF have on growth, nutritional status, and hormone levels of inoculated plants, *Triticum aestivum* and *Triticum durum*. Three inoculation methods were used using a conidial suspension of *B. bassiana* (Balsamo) Vuill with a concentration of 10⁸ conidia mL⁻¹, soil treatment, seed dressing and leaf spraying (2 first leaves of wheat plants 7 days after germination), with 25 plants per treatment either treated / inoculated or control. Plant growth parameters were determined and evolution of the fungal inoculum in the soil and colonisation of plant tissues (leaves and roots) assessed through re-isolation of *B. bassiana* at different phenological states. The fungus was revealed to be rhizosphere-competent, with root re-isolation percentages ranging from 20 to 80% for plants grown on soil treatment and seed dressing. Percentage of fungal re-isolation from leaf tissues was significantly higher in plants inoculated by leaf spraying ranging between 8 and 75 %. At the end of the crops, it was detected that the dry weight, the total root length, the quantity of some nutrients and yield of inoculated plants was higher than in control plants. The possible origin of these differences in *B. bassiana* inoculated plants and their implications for pest and disease control and the promotion of plant growth are being investigated.

Poster / Fungi. Wednesday, 16:30. **FU-11-STU**

Interactions between cowpea plants vs. *Metarhizium* spp. entomopathogenic fungi

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In recent years, *Metarhizium* spp. fungi have been reported to associate with plants through rhizosphere competence and endophytic growth. Benefits to both the fungus and the plant, at least in some cases, is beneficial to both members of the pair. In the present study, germination of two important *Metarhizium* species was analyzed after incubation of conidia on young plant leaves. Seeds of *Vigna unguiculata* (cowpea) were planted in sterile soil and incubated with ambient light at room temperature for 10 days. Aqueous suspensions of *Metarhizium robertsii* ARSEF 2575 or *Metarhizium brunneum* ARSEF 1095 were brushed directly onto plant leaves. Control plants, to ensure conidial germination, had their leaves brushed with potato dextrose agar (PDA) plus 0.05% chloramphenicol and 0.002%

benomyl; air dried; then the fungus suspension was brushed on the leaves. After 24h and 48h, 0.5 cm² leaf pieces were examined by scanning electron microscopy (SEM). Conidia of both fungal isolates germinated on cowpea leaves 24h and 48h after inoculation on both PDA treated (control) and PDA not treated (test) leaves. SEM observation showed conidial adherence but with no preferred attachment sites. Each conidium produced one germ tube; and both long and short germ tubes were observed. Their growth over the plant cuticle was random (had no apparent targets). There was no evidence of appressorium formation. However, some *M. brunneum* ARSEF 1095 conidia that germinated on non-PDA-treated leaves had images that suggested direct penetration. Culture studies with surface-sterilized fungus-exposed leaves are underway to verify or deny cuticular penetration.

Poster / Fungi. Wednesday, 16:30. **FU-12**

Biological control in oilseed rape: An attempt to establish the entomopathogenic fungus *Beauveria bassiana* as an endophyte in oilseed rape plants

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With the rapid spreading of the cultivation of oilseed rape (*Brassica napus* L.), the populations of pest insects of rapeseed also increase, in particular the rapeseed pollen beetle (*Brassicogethes aeneus*) and rape stem weevil (*Ceutorhynchus napi*). Hence, the aim of the investigations within the scope of biological control is to establish the entomopathogenic fungus *Beauveria bassiana* Naturalis ATCC74040 as a systemic endophyte in oilseed rape. Blastospores of *B. bassiana* (10⁵ Sp/ml) from Czapek liquid medium were infiltrated into rape leaves. The plants were held with 80% RH and 20°C on long day conditions. Between 3 days and 4 weeks leave samples were taken and examined by fluorescence-microscopy, either with Blankophor or specifically with polyclonal primary antibodies against *B. bassiana*. PCR primers targeting a characteristic partial sequence of a self splicing group-I intron within the 28S rRNA encoding gene of *B. bassiana* Naturalis ATCC74040 were designed and used for strain-specific diagnosis. While the fungus was found to be persistent on the epidermis, only few hyphae could be detected microscopically in intercellular space of the leaves. By means of PCR, *B. bassiana* Naturalis could be proven successfully in rape tissue samples; a clear molecular proof of systemic growth within leaves is still pending. Possible defense mechanisms are discussed.

Poster / Fungi. Wednesday, 16:30. **FU-13**

Azygo- and zygosporangium formation of *Neozygites floridana* in the two-spotted spider mite (*Tetranychus urticae*) in strains from tropical and temperate regions

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Neozygites floridana is an obligate fungal pathogen of mites in the family Tetranychidae and is an important natural enemy of the two-spotted spider mite (*Tetranychus urticae*). Until now, information about the formation of azygospores remained to be fully confirmed. In this study, we document the formation of

azygospores by a Brazilian *N. floridana* strain and the formation of azygospores and zygozospores by a Norwegian *N. floridana* strain both in the host *T. urticae*. Evidence of both zygozosporegenesis and azygozosporegenesis was also found in the same individual in the Norwegian strains. Further we report the presence of immature azygospores with 1-3 nuclei for the Norwegian strains, immature resting spores (probably azygospores) with 1-8 nuclei for the Brazilian strain, and mature resting spores with 2 nuclei for both the Norwegian and the Brazilian strains (azygo- or zygozospores). Our observations suggest that the immature resting spore (prespore) of both strains begins in a multinucleate condition but that the nuclear number is reduced during maturation until mature resting spore is binucleate regardless of its origin as zygozospore or azygozospore.

Poster / Fungi. Wednesday, 16:30. **FU-14**

Susceptibility of *Biomphalaria glabrata* egg masses to fungal infection

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Aquatic *Biomphalaria glabrata* snails from the neotropics are most common in stagnant or slow-flowing water habitats. Quantities of egg masses are laid near the water surface on submerged substrates but are often eventually exposed to desiccation and natural enemies. Almost nothing is known about fungal pathogens acting against these snails. We report on the ovicidal activity of *Metarhizium anisopliae* (IP 46) and *Beauveria bassiana* (ARSEF 9588). Freshly laid egg masses (5 masses each test of four independent repetitions) were either exposed to water and treated with 2×10^7 conidia or hyphal bodies/ml of these fungi or treated topically (2×10^7 conidia or hyphal bodies) and then incubated in a permanent water film in a moist chamber at 25°C. Controls were treated with water only. Egg masses were checked daily for fungal growth and eclosion of juveniles. After application of conidia or hyphal bodies, IP 46 developed distinct mycelium and new conidia on egg masses in water film, and hyphal bodies yielded no later eclosion of juveniles. No mycelium developed when ARSEF 9588 was applied to egg masses exposed in water films and all juveniles eclosed. In water, both fungi developed mycelium after application of conidia or hyphal bodies to egg masses, and juveniles failed to eclose. All juveniles eclosed from uninoculated egg masses exposed in water or film. The results suggest that both *M. anisopliae* and *B. bassiana* may act against *B. glabrata* egg masses, but that the degree of molluscicidal activity depends on the type of fungal inoculum applied.

Poster / Fungi. Wednesday, 16:30. **FU-15**

Antimicrobial, Antioxidant and Anticancer Activity of Culture Filtrates from Entomopathogenic Fungi

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Entomopathogenic fungi are natural pathogens of insects and contribute to the regulation of host insect populations in the environment. Several of these fungi produce a wide range of secreted enzymes, secreted protein toxins and secondary metabolites to overcome host defenses and ultimately kill the host, and to defend host resources against competing pathogens and saprophytes. Therefore, this study was performed to select

the antimicrobial activity of entomopathogenic fungi from Korea soils against plant pathogenic bacterium *Ralstonia solanacearum* and plant pathogenic fungus *Botrytis cinerea* using dual culture technique on SDYA. In addition, we also performed to screening of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals scavenging activity compounds from liquid culture filtrates of entomopathogenic fungi and investigate to its anticancer activity. As results, 12 isolates, 6 isolates and 25 isolates showing of these fungal metabolites produced antibacterial, antifungal and radicals scavenging activity compounds, respectively. The preferential antimicrobial, radical scavenging and anticancer activities give evidence that these entomopathogenic fungal metabolites might be useful as a source for plant pathogen control and pharmaceutical interests.

Poster / Fungi. Wednesday, 16:30. **FU-16**

Evolutionary-ecological strategies of *Metarhizium robertsii*

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The species of the entomopathogenic fungus *Metarhizium* include forms characterized by different pathogenic strategies. Two strains of entomopathogenic fungus *M. robertsii* with different strategies were investigated. The strain Mak-1 («growth strategy») is characterized by slow killing speed of different insect species (Orthoptera, Coleoptera, Diptera, Lepidoptera) and abundant sporulation on cadavers. The strain P-72 («toxin strategy») is characterized by significantly rapid killing speed, but sporulation of this strain was detected only on Lepidoptera. Thus the fungi specialization can be associated with necrotrophic (but not biotrophic) phase of life cycle. In addition P-72 is characterized by the higher level of destruxin B, E production, rapid activation of conidia on the artificial media and insect's cuticle. The strain P-72 was more productive in media from plant compounds while Mak-1 - on insects and media of them. Our results show that «non-toxicogenic» strain has higher adaptation to entomoparasitic nutrition, and the «toxicogenic» strain to saprophytic nutrition. We found the change of the defense systems of Colorado potato beetle (*Leptinotarsa decemlineata*) larva (increasing of phenoloxidase in cuticle and detoxificative enzyme in fat body and hemolymph, decreased rate of cells immunity) under infection by the «toxicogenic» strain but not by strain with «growth strategy». Our data support hypothesis that evolution of entomopathogenic fungi *Metarhizium* was directed with a loss association with plants and formation of specialized entomoparasitic forms.

Poster / Fungi. Wednesday, 16:30. **FU-17**

Mycelial and conidial thermotolerance of *Metarhizium anisopliae* s.l. IP 46 and *Metarhizium robertsii* ARSEF 2575

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High temperature is a very important environmental stressor that may limit efficacy of fungi in arthropod biocontrol programs; however, formulation of fungal propagules is suggested for increasing performance of fungi. The current study was designed to evaluate the radial growth of colonies of *Metarhizium anisopliae* s.l. IP 46 and *M. robertsii* ARSEF 2575 on PDAY

culture medium incubated at 27±1°C (optimum) or 32±0.5°C (heat stress) for 15 days. Colonies diameter was measured daily, and at day 15 the conidia produced were quantified, and their viability assessed. In addition, thermotolerance of conidia prepared in different additives was investigated; accordingly, dried conidia were suspended in water solution (Tween 80, 0.01%), commercial emulsifiable or non-emulsifiable oils or carboxymethyl-cellulose gel (CMC), and exposed to 45±0.5°C for 4, 6 or 8h. Germination was assessed 48h after inoculation of conidia onto PDAY plates. A significant reduced radial growth and conidial production were shown in colonies incubated at 32±0.5°C, but conidial viability was high (>98%) for both fungi grown under optimum or heat-stressed conditions. Viability of conidia suspended in water solution, commercial emulsifiable oils or CMC, and exposed to 45±0.5°C was drastically low [0% mean relative germination (RG) at 8h exposure]. Conversely, conidia suspended in non-emulsifiable canola or mineral oil had high viability (69.3% and 71.8% RG for ARSEF 2575, and 95.0% and 80.2% RG for IP 46, respectively, both at 8h exposure). In conclusion, oil formulation minimizes the effects of high temperature to conidia of these entomopathogenic fungi, indicating that conidia applied to the field could persist longer in heat-stressed environments and that their development may occur during periods reaching optimum temperatures.

Poster / Fungi. Wednesday, 16:30. **FU-18**

Delayed germination of heat-stressed conidia of *Metarhizium anisopliae* on tick cuticle

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The current study assessed the germination of heat-stressed conidia of *Metarhizium anisopliae* IP 119 on cuticle of *Rhipicephalus sanguineus*. Aqueous conidial suspensions (Tween 80, 0.01%) of *M. anisopliae* IP119 were exposed to 0 h (non-heated control) or 4 h at 45±0.5°C (heat-treated test) in a water bath, and then inoculated onto either the dorsal surface of *R. sanguineus* engorged females or onto PDAY culture medium. The samples were incubated at 27±1°C and RH>80% for 0, 12, 18, 24, 36, 48 or 72 h. After each incubation time, ticks were dissected, i.e., the dorsal cuticle was removed and immersed in Calcofluor White 2% overnight, then observed by fluorescence microscopy for evaluation of conidial germination. Conidial germination on PDAY plates was assessed using a phase-contrast microscope. A minimum of 300 conidia per cuticle or plate was evaluated, and percent germination calculated. It was found that conidial germination on tick cuticle was delayed in comparison to germination on artificial culture medium, regardless the incubation time. When conidia were exposed to heat, a higher percent germination was detected on PDAY (61.5%) in comparison to the tick cuticle (13%) at 72 h after inoculation. On tick cuticle, appressoria from non-heated (control) conidia were observed 36 h after inoculation, whereas no appressoria were seen from heated conidia (test) at any incubation period after inoculation, including 72 h. In conclusion, heated conidia germinated faster when they were inoculated on PDAY than when they were applied to the tick cuticle. This result suggests that the negative effect of heat on conidial germination was greater when the conidia were applied to arthropod cuticle than would be predicted by *in vitro* (artificial medium) thermotolerance tests. In addition, the technique of fluorescence microscopy proved to be a simple method for visualizing germinated conidia and appressoria on the cuticle of *R. sanguineus*.

Poster / Fungi. Wednesday, 16:30. **FU-19**

Influence of environmental factors on insects resistance to anamorphic fungi

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We studied effect of different factors such as suboptimal temperatures, sublethal bacterial infection (*Bacillus thuringiensis*), synthetically and natural insecticides (pirimiphos-methyl, *Cordyceps militaris*) and venom of parasitoid *Habrobracon hebetor* on defense systems of wax moth *Galleria mellonella* and Colorado potato beetle *Leptinotarsa decemlineata*. Moreover insect susceptibility to fungi *Beauveria bassiana* and *Metarhizium robertsii* under these factors has been examined. We found the decreasing of phenoloxidase activity in hemolymph and cuticle, and detoxicative enzymes activity (nonspecific esterases, glutathion-S-transferases) in hemolymph, as well as in encapsulation response. Thus dramatic depression in host's defense systems led to increased susceptibility of insects to fungi from ten to several thousand times. Our data support hypothesis that low specificity of anamorphic entomopathogenic fungi is closely associated with their ability to infect insects with defense system seriously suppressed by various environmental factors.

Poster / Fungi. Wednesday, 16:30. **FU-20**

Intraspecific and interspecific variation in osmotolerance of entomopathogenic fungi

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Entomopathogenic fungi must be capable of cell division under multiple stresses imposed during the various stages of the lifecycle, some of which take place on the insect surface or within the hemolymph. These include the energy-expensive synthesis and retention of compatible solutes to maintain osmotic pressure. The windows for osmotolerance of 24 isolates of entomopathogenic fungi were determined by assessing conidial germination over a range of KCl concentrations. Germination was evaluated on potato dextrose agar (PDA; control) or PDA+KCl using 31 concentrations of KCl from 100 to 3000 mM (after 24 h; 26 °C). *Trichothecium roseum* was the most osmotolerant (≤ 3000 mM KCl), followed by *Lecanicillium aphanocladii*, *Simplicillium lanosoniveum*, and *Isaria fumosorosea*. Several fungal species showed moderate osmotolerance (≤1700 mM) including *Metarhizium robertsii* (for some isolates), *Metarhizium brunneum*, *Metarhizium anisopliae*, *Tolypocladium inflatum*, *Tolypocladium cylindrosporium*, and *Fusarium coccophilum*. Some isolates showed modest levels of osmotolerance (≤ 1400 mM), including one isolate of *M. robertsii*, one of *M. anisopliae*, two of *M. acridum*, and *Beauveria bassiana*. *Aschersonia aleyrodis* and one isolate of *M. brunneum* were relatively intolerant to osmotic stress (≤ 1000 mM KCl). These findings indicate high levels of inter- and intraspecific variability in osmotolerance for insect-pathogenic fungi. Eighty percent of *Trichothecium roseum* conidia germinated at 2000 mM KCl (equivalent to 0.928 water activity), with a LC50 at 2300 mM, and some germination at < 0.890 water activity (on 3000 mM KCl). This suggests that *T. roseum* is highly xerotolerant and may therefore be unique amongst the entomopathogenic fungi.

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Poster / Fungi. Wednesday, 16:30. **FU-21**

Different intensities of visible light during mycelial growth induce differently the conidial tolerance to menadione in *Metarhizium robertsii*.

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The threshold of illumination during mycelial growth influenced the conidial tolerance of an entomopathogenic fungus to the oxidative agent menadione. *Metarhizium robertsii* (ARSEF 2575) was grown at 26 °C for 14 days in five treatments: 1) minimal medium (MM) in the dark; 2) potato dextrose agar (PDA) in the dark inside the Panasonic incubator; 3) PDA medium under continuous visible light in the Panasonic incubator; 4) PDA medium in the dark inside the Marconi incubator; 5) PDA medium under continuous visible light inside the Marconi incubator. For the Panasonic incubator, three intensities of light were studied with 1, 3, and 5 lumens. The germination of conidia produced under these treatments was subsequently evaluated on PDA medium supplemented with menadione at the concentrations 0.10 and 0.15 mM. For control, conidia germinated on PDA medium. The germination was evaluated counting at least 300 conidia after 24 h at 26 °C. Each treatment was repeated four times with a new batch of conidia produced for each repetition. Conidia produced on minimal medium were more tolerant to menadione, followed by conidia produced under visible light inside the Marconi incubator. Conidia produced inside the Panasonic incubator at 5 lumens were more tolerant to menadione, but less tolerant than conidia produced under light in the Marconi incubator. Conidia produced in the Panasonic incubator at 1 and 3 lumens showed somewhat increased tolerance as compared with control in the dark. Therefore, growth under visible light produced conidia more tolerant to menadione.

Poster / Fungi. Wednesday, 16:30. **FU-22**

Effect of *Metarhizium* spp. growth media on the accumulation of destruxins in a 10-L stirred tank reactor

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Entomopathogenic fungi secrete a wide range of metabolites, mostly products of secondary metabolism. These metabolites serve different functions depending on the ecological niche of the fungus. Nevertheless, the EU-approach to microbial metabolites is still under discussion and therefore, three *Metarhizium brunneum* production strains were assessed for their secondary metabolite secretion (i.e. destruxin A, B and E) in a 10-L stirred tank reactor. Biomass production on the Sabourand-4 glucose - complete medium - and on a modified Czabek-Dox media, blended with yeast extract without peptone was tested two-times in batch-

fermentation runs. The aim was to figure out whether secondary metabolite impurities (i.e. destruxin analytes) in the technical BCA products derive from a overdosage of complex nutrient ingredients or if they are routinely formed during the BCA production process. The destruxin A, B and E accumulation considerably decreased for all three production strains by avoiding peptone as nitrogen source. Comparing the three production strains in both culture broth batch-systems it must be concluded that the strains differ in the amount of destruxin accumulation. Crude extract products are now available for the purpose of further risk assessment studies of *Metarhizium* metabolites (a.o. cytotoxicity and genotoxicity studies).

Poster / Fungi. Wednesday, 16:30. **FU-23**

Evaluation of destruxin A production in four strains of *Metarhizium* by capillary electrophoresis

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Destruxin A (dtx A) is one of the main secondary metabolites produced by *Metarhizium* strains that exhibit insecticidal activity. Due to its toxicity, and the fact that it could be a risk to humans and the environment since it is able to enter in the food chain, the interest in learning more about the detection of this metabolite has increased in recent years. In this study the production of dtx A by four different strains (BIPESCO5, EAMA 01/58-Su, ART 2825 and ARSEF 23) was evaluated. These strains were grown in four different culture mediums (CM: semi-synthetic complete medium; MM: minimal medium; OSM: osmotic stress medium; CN2: peptone in water). All analyses were carried out using a powerful separation technique named Capillary Electrophoresis with Ultraviolet detection (CE-UV). The results showed that ARSEF 23 cultivated in MM medium was the only strain which produced dtx A with a maximum concentration of 20.2 mg/L. In CM medium, BIPESCO5, ARSEF 23 and EAMA 01/58-su strains produced dtx A at different concentrations (24.4 mg/L, 9.9 mg/L and 7.8 mg/L, respectively). Under the CE conditions selected, dtxA was not detected in ART 2825 strain. No strains cultivated in either OSM or CN2 medium produced detectable amounts of dtxA. Our results indicate that the production of dtx A by strains depends on the culture medium, probably related to glucose content. Additionally, it can be confirmed that CE coupled with UV detector is a suitable tool to identify and quantify dtx A (at concentrations higher than 0.5 mg/L) in fungal culture medium.

Poster / Fungi. Wednesday, 16:30. **FU-24**

Entomopathogenic fungal genera and the 1F=1N standard: The shape of the future begins to emerge

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Recent changes to the International Code of Nomenclature for algae, fungi and plants compel changes in how pleomorphic fungal genera are named, and they disallow the retention of separate generic names for sexual and asexual morphs of the same taxa. These changes broadly affect many fungi, but

strongly affect the taxonomically complex insect pathogens of Hypocreales. Molecular data and phylogenetic reconstructions are being used to develop community-driven, consensus-based proposals for conservation and rejection of generic names for the affected fungi. These efforts seek to stabilize generic concepts around well supported monophyletic clades while minimizing disruption to the diverse research and user communities dealing with these fungi. Inevitably, some widely studied genera will be synonymized, and their names will no longer be available except in a descriptive manner (e.g., hirsutelloid morphology rather than *Hirsutella*). Proposals for Ophiocordycipitaceae and some taxa in Clavicipitaceae (notably *Metarhizium* and closely related genera) are now available. The current draft proposal for genera of Cordycipitaceae is presented here. Despite the substantial effort involved in generating the lists presented here, real challenges in resolving some relationships remain; future studies can be expected to justify the recognition of still more segregate genera than are now listed in the proposals. The senior author will continue to update the SIP membership about relevant changes at future SIP meetings and the ARSEF collection's website (<http://www.ars.usda.gov/Main/docs.htm?docid=12125>).

Poster / Fungi. Wednesday, 16:30. **FU-25**

Genotyping of Georgian isolates of entomopathogenic fungi *Beauveria* spp.

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Our research is about genotyping different subspecies isolates of *Beauveria* collected from various regions of Georgia. *Beauveria* spp is one of the most widely spread entomopathogenic fungi in agriculture. It is a producer of toxins and biological active materials, which can cause high mortality in different species of pests. Nowadays, there is high interest towards active strains of *Beauveria*. Use of molecular biology techniques has demonstrated that *Beauveria* spp (7 isolates from different habitats and geographical zones of Georgia) unites unknown species and their determination by traditional conidial morphology is impossible. We have done phylogenetic characterization of *Beauveria Bassiana*: (I) Polymerase Chain reaction (PCR) to differentiate the clades of Georgian strains (It has never been investigated to which clades A, B or C they belong); (II) Sequencing of DNA fragments from ITS region (the rRNA gene cluster) and (the Elongation Factor 1-alpha) EF1 and (the intergenic) Bloc region. At present, we plan to identify proteins that are responsible for the virulence of *Beauveria Bassiana*. This study gives us opportunity to understand population of *Beauveria* and its future applications in effective biocontrol strategy of pathogens. Attention to biocontrol is a breath taking perspectives for sustainable development of the world.

Poster / Fungi. Wednesday, 16:30. **FU-26**

Genetic characterization, fungicide sensitivity, and aphicidal potential of *Lecanicillium* fungi from Argentina

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Mitosporic fungi of the genus *Lecanicillium* (Ascomycota; Hypocreales) are of particular interest as biological control agents for phloem-sucking plant pests including aphids. Bioprospection for these fungi in Argentina has given rise to a set of single-spore derived *Lecanicillium* strains isolated from a wide range of original hosts. Current species delineation within the taxonomic genus *Lecanicillium* that consists of the three "core species" *Lecanicillium lecanii*, *L. muscarium*, and *L. longisporum* as well as further less closely related species, is not free of ambiguity. For species-level characterization of *Lecanicillium* isolates, a set of five genetic markers comprising one mitochondrial (NMS) and two nuclear (ITS, IGS) ribosomal RNA operon together with one mitochondrial (*nad1*) and one nuclear (*ef1α*) protein-encoding sequences, has been employed. The aggregated information from these markers indicates that fungal isolates from Argentina mainly, but not exclusively belong to the *Lecanicillium* core species. Moreover, the set of *Lecanicillium* strains has been investigated for fungicide sensitivity. Between strain differences in susceptibilities have been found to be important and not necessarily in line with systematics, making careful determination of sensitivity to agriculturally used fungicides an important criterion of biocontrol agent selection. However, the fungicidal polyketide compound soraphen has been found of outstanding activity against a wide variety of isolates from all species investigated. On the basis of these results, a subset of strains has been selected for virulence bioassays against the green peach aphid, *Myzus persicae*, an important agricultural pest in Argentina and other parts of the world.

Poster / Fungi. Wednesday, 16:30. **FU-27**

Species-specific PCR assay to identify and discriminate *M. pingshaense*, *M. anisopliae*, *M. brunneum*, and *M. robertsii*

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Metarhizium comprises important fungal pathogens of insects and several species in the *M. anisopliae* complex are in use for biological control of insect pests. The most recent taxonomic revision of the *M. anisopliae* species complex used a multilocus phylogenetic (EF-1α, RPB1, RPB2 & β-tubulin) approach and nine species are now recognized. Accurate molecular identification of these species is possible using the 5' region of EF-1α or one of seven recently developed nuclear intergenic loci. The goal of this study was to develop a species-specific PCR assay to rapidly identify species of the "PARB" clade, which includes *M. pingshaense*, *M. anisopliae*, *M. robertsii* and *M. brunneum*, without the need to obtain full-length sequence reads. Markers included in the recent multilocus phylogeny (ITS, rIGS, EF1-α, EF1-5', RPB1, RPB2 and β-tubulin) and 5 nuclear intergenic (nuclIGS) sequence markers for *Metarhizium* were screened for the presence of species-specific sequence signatures amenable for discriminatory PCR primer design. One primer pair was designed each for *M. anisopliae* (rIGS), *M. robertsii* (rIGS) and *M. pingshaense* (MzIGS2), and two primer pairs were designed for *M. brunneum* (both MzIGS2). Specificity

of the different primer pairs was tested by performing BLAST similarity searches and PCR amplifications on a collection of 65 strains representing 11 different *Metarhizium* species. The approach was further validated by identifying soil isolates collected from a Swiss meadow.

Poster / Fungi. Wednesday, 16:30. **FU-28**

Species identification of entomopathogenic fungi of the genus *Lecanicillium* (= *Verticillium lecanii* s.l.) by mitochondrial gene sequences

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For species identification of entomopathogenic fungi of the genus *Lecanicillium* (former *Verticillium lecanii* Zimm. Viegas) from collection of All-Russian Institute of Plant Protection, sequencing of mitochondrial gene *nad1* was exploited. Among 39 isolates, 36 showed attribution to *Lecanicillium muscarium*, 2 – to *Lecanicillium psalliotae* and one – to *Lecanicillium longisporum*. In *Lecanicillium muscarium*, 4 *nad1* molecular haplotypes were detected. Only one of them was identical to that already present in Genbank (EF512920). Two novel haplotypes were 99.3-99.7% similar to each other and to the former haplotype. Finally, the fourth haplotype was similar to the other three at the level of 97.9% sequence similarity and was represented by 14% of the isolates under study. The geographic origin and isolation source (partially reflecting the host specificity) were diverse with no consistent pattern among haplotypes. Supported by RFBR # 13-04-01905.

Poster / Fungi. Wednesday, 16:30. **FU-29**

The genomic basis for evolved resistance to *Beauveria bassiana* in *Drosophila melanogaster*

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We use an “evolve and resequence” approach to determine the genomic basis for evolved resistance to the fungal pathogen *Beauveria bassiana* in the genetic model insect, *Drosophila melanogaster*. Entomopathogenic fungi, such as *B. bassiana*, are used in biological control of mosquito vectors of dengue fever and malaria, and of various agricultural insect pests. To better understand mechanisms of insect resistance to *B. bassiana*, we artificially select *D. melanogaster* for increased resistance to this pathogen in very large, replicated experimental populations. The populations that are selected for increased resistance to *B. bassiana* have not evolved cross-resistance to bacterial pathogens, which suggests that selection may be acting on mechanisms outside of core immunity. We genotype the selected and control populations at multiple generations throughout selection to identify relevant genes and to make inferences about the temporal trajectories of adaptive alleles. We are developing novel methods for analysis of pooled sequences from such evolve and resequence datasets that will provide better assessment of technical artifacts and accurately identify regions of the genome that have responded to selection.

Poster / Fungi. Wednesday, 16:30. **FU-30-STU**

Behavioral control of malarial mosquito by entomopathogenic fungi: Death as the vector

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Our previous study clarified infection of entomopathogenic fungi via the proboscis route is important on rapid mosquito death rather than infection route from tarsomere, and death of mosquito highly correlated with fungal invasion to brain. We developed a hypothesis that fungal infection via proboscis route can affect to mosquito behavior, and the aim of this study was to investigate the alteration of host searching behavior of mosquito by entomopathogenic fungi infection until the mosquito dies.

The mosquitoes were inoculated with *B. bassiana* s.l. 60-2, and quantification of the total amount of host searching behavior in a free flight system by using automated-recording device was conducted. Attractiveness of fungus infected mosquitoes and mock mosquitoes to the heat (40°C) and the color (black) were evaluated in this device for 10 days. As a result, attractiveness to the heat was drastically decreased from 3 days post inoculation, whereas attractiveness to the color has a tendency to decrease from 6 days post inoculation. This reduction of response to mosquito attractant might be caused by fungal infection to their head where has various important sense organ to search host. It will inhibit or damage to their heat and visual sensors or sensory neuron, then mosquitoes became less able to recognize host cues (death as the vector). Although conventional vector control has only focused on killing vectors, our results indicate that there need holistic evaluation as disease transmission risk on vector control using entomopathogenic fungi including lethal and sub-lethal effects.

Poster / Fungi. Wednesday, 16:30. **FU-31**

Effect of *Metarhizium brunneum* strain LRC112 and *M. anisopliae* F52 on non-target Carabid Beetles

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Carabid beetles are considered to be among the most important beneficial insects in agricultural ecosystems and are commonly associated with agricultural fields in British Columbia. Sustainable treatment to control click beetles and wireworms should have little impact on non-target carabid beetle populations. In the present study, we examined the effect of the *M. brunneum* strain LRC112 on adult *Agriotes obscurus* and on common local Carabid species. Further, we compared the effects of *M. brunneum* strain LRC112 to the commercial *M. anisopliae* F52 strain. Examined Carabid beetle species were less susceptible to the tested *Metarhizium* strains than *A. obscurus* beetles. Additional assays at multiple spore concentrations of both *Metarhizium* strains were conducted on two common Carabid species: *Pterostichus melanarius* and *Calathus fuscipes*. For both beetle species, significant mortality was observed at the highest *M. anisopliae* F52 concentration, whereas little mortality was observed at the highest *M. brunneum* LRC112 concentration.

Poster / Fungi. Wednesday, 16:30. **FU-32**

Effect of a local strain of the fungus against *Corythucha ciliata* (Say) and *Glyphodes pyloalis* (Walker) in Georgia

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The sycamore lace bug, *C. ciliata* is one of the most destructive pest of plane trees (*Platanus* spp.) all over the world. This pest is also known to be major nuisances in Georgia since plane trees has been very popular in parks and planting of the cities. The lesser mulberry pyralid, *G. pyloalis* which was spread and caused damage to *Morus alba* in recent years in Georgia is very big problem as well.

Isaria fumosorosea isolated from pupae of *Hyphantria cunea* Drury in Georgia was evaluated to determine its potential as a biological control agent of these pests. Second and third instar larvae of *G. pyloalis* were collected in Tbilisi from *Morus alba* trees and *C. ciliata* adults were collected from the bark of *Platanus* trees in Kutaisi, Georgia. A conidial suspension, concentration 10⁹ conidia/ml, was used for both experiments. The suspension was applied to bark to expose *C. ciliata* adults and *M. alba* leaves to expose *G. pyloalis* larvae under laboratory conditions. Efficacy, corrected with mortality in the control treatment, was calculated according Schneider-Orelli's formula. *I. fumosorosea* showed 30% corrected efficacy against larvae of *G. pyloalis*, and 50% for *C. ciliata*. The results of this study suggest that larvae of *G. pyloalis* were tolerant to the induced mycoses caused by *I. fumosorosea*, but more effect on the mortality of *C. ciliata* adults. Experiments are needed to determine the IC₅₀ of the fungus for the two pests and to develop appropriate application methods if efficacy proves to be sufficient.

Poster / Fungi. Wednesday, 16:30. **FU-33**

The effect of pesticides used in strawberry and soybean on the mite pathogenic fungus *Neozygites floridana*

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Neozygites floridana is an important natural enemy of the two-spotted spider mite, *Tetranychus urticae*. Pesticides used in strawberry and soybean that might affect the conservation and enhancement of this beneficial fungus in Integrated Pest Management (IPM) systems were therefore studied in laboratory. Eighteen pesticides were sprayed on mummified mites killed by the *N. floridana* isolate ESALQ1420 placed on coverslips with alphanumeric coded squares. The effect of these pesticides on the sporulation and capilliconidia production (germination) of *N. floridana* were determined. Recommended concentrations (RC) and half of this concentration (RC/2) were used, and the control was sprayed with distilled water plus 0.05 % Tween 80. The treated cadavers were set to sporulate for 12h in darkness at 25±2°C and 100% RH. The acaricide Vertimec (Abamectin) at half dose resulted in a primary conidia production of 1283(±169) and 38%(±12) of these produced capilliconidia (germinated). RC of Folicur (Tebuconazol) resulted in a primary conidia production of 1558(±308) and 37%(±11) germination. Further, RC of the insecticide Danimen (Fenpropratin), resulted in a primary conidia production of 1057 (± 201) and a resulting 37%(±11) germination. RC of Talcord (Permethrin) resulted in 1292 (±335) primary

conidia and 74%(±4) germination and Karate at RC/2 in 2985(± 337) primary conidia and 83%(±) germination. This demonstrates that Vertimec, Folicur, Danimen, Talcord and Karate (Lambda-cyhalothrin) were the five pesticides that had the lowest impact on *N. floridana*. Products containing sulfur even in RC/2 were detrimental to *N. floridana*. Thiovit Jet (sulfur) resulted in a primary conidia production of only 162(±84) and 0%(±0) germination and no sporulation was observed from mummified mites sprayed with Kumulus (sulfur). These results are important considering that organic farmers extensively use sulfur-based products in order to control phytopathogenic fungi.

Poster / Fungi. Wednesday, 16:30. **FU-34**

Development of a granular formulation of *Metarhizium brunneum* based on mycelial fragments

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The application of the entomopathogenic fungus *Metarhizium brunneum* strain Ma43 (=BIPESCO 5 = F52) against soil dwelling pests like *Otiorynchus sulcatus* needs specific requirements on the product. Although the fungus can be grown on solid media the fermentation time in solid state fermenter is long and labor intensive. Additionally, problems with the application of the fermented grain are reported. Therefore, we investigated the possibility of formulating mycelial fragments. Mycelium of Ma 43 was produced in a liquid fermenter and was homogenized to get a flowable suspension. The results demonstrate that humid heat of up to 70° C reduce the viability of the mycelial fragments whereas dry heat of up to 70° C did not influence the viability. Further experiments on fluid bed drying demonstrated that mycelial fragments can be coated on millet at temperatures of 50° C. After coating the fungus was growing and sporulating on the surface of the millet under humid conditions. Protectants like lactose enhanced the viability after fluid bed drying. Further optimization steps and the practicability of mycelial fragments based formulations will be discussed.

Poster / Fungi. Wednesday, 16:30. **FU-35**

Innovative biological products for soil pest control: Outline of an EU project

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Many herbivorous insect pests have soil dwelling larval stages, which are difficult to control. These subterranean insect pests, such as the western corn rootworm, wireworms, black wine weevil, scarids, white grubs, and tipulids, currently need to be controlled by insecticidal applications. However, in complying with EU directives, several pesticides are or will be phased out in the near future, requesting new and complementary control

strategies. INBISOIL explores in detail the recently discovered synergistic effects between entomopathogenic fungi (EPFs), entomopathogenic nematodes (EPNs), and semiochemicals by developing innovative co-formulations, making use of strategies derived from nature. These co-formulations will be based on capsules containing EPFs (*Metarhizium brunneum* or *Beauveria bassiana*) in combination with strains of EPNs (*Heterorhabditis bacteriophora*), or semiochemicals. Additionally, INBISOIL will develop integrated pest management (IPM) strategies that exploit synergies between these biocontrol agents and semiochemicals. The overall aim of the project INBISOIL is to optimize the use of biocontrol agents in the soil for more efficacious, low input, control of pests in farming systems of major importance in Europe. New crop protection strategies will be developed that will i) reduce pesticide inputs, ii) provide protection in non-sterile soils, eliminating for soil sterilants, iii) reduce production costs, and iv) result in the production of high-quality and safer crops in accordance with theme priority area (Integrated pest management in farming systems of major importance in Europe).

Poster / Fungi. Wednesday, 16:30. **FU-36**

Oxidative stress levels in the entomopathogenic fungus *Beauveria bassiana* growing in very long-chain hydrocarbons

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Broad host range entomopathogenic fungi attack insect hosts via attachment to insect surface, with the subsequent production of degrading enzymes that help penetration through the cuticle. The outermost insect surface is covered by a lipid-rich layer, usually composed of very long-chain hydrocarbons. It is known that *B. bassiana* is able to grow on straight chain hydrocarbons (alkanes) as a sole source of carbon and energy, but it would have to pay a high cost to do so. The aim of this work was to study the oxidative stress levels in alkane-grown *B. bassiana*. For this purpose, we analyzed the gene expression pattern of *sod1*, *sod2*, and *sod3* encoding superoxide dismutases, *catA*, *catB*, *catC*, *catD*, and *catP* encoding catalases, and *gpx* encoding glutathione peroxidase; and the enzymatic activity of SOD, CAT, and GPx in crude homogenates. Fungi grown either in hexadecane (*n*-C16) or octacosane (*n*-C28) showed overlapping but differential gene induction, with a concomitant increment in enzymatic specific activities, compared with controls grown in complete medium. These results confirm that high levels of reactive oxygen species are produced in *B. bassiana* during growth in alkanes, and an antioxidant response is triggered in fungal cells to overcome this drawback.

MICROBIAL CONTROL

Poster / Microbial Control. Wednesday, 16:30. **MC-1-STU**

Fungal strain selection and screenhouse evaluation of the virulent isolate against aphids on crucifer and okra vegetables

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Aphids are major pest problems of crucifer and okra vegetables in sub-Saharan Africa. Biopesticides are now acceptable pest control alternatives to synthetic chemical insecticides. Five isolates of *Metarhizium anisopliae* and three of *Beauveria bassiana* were screened for virulence against the following apterous adult aphids in the laboratory: *Brevicoryne brassicae* and *Lipaphis pseudobrassicae* on kale, and *Aphis gossypii* on okra. *Metarhizium anisopliae* isolates ICIPE 30, ICIPE 62 and ICIPE 69 outperformed the others causing mortality of 85-98%, 83-97%, and 73-77%, in *B. brassicae*, *L. pseudobrassicae* and *A. gossypii*, respectively, at 5 d post inoculation. However, *M. anisopliae* ICIPE 62 had the shortest LT₅₀ values of 2.8, 2.1 and 1.9 d; and the lowest LC₅₀ values of 5.5×10⁵, 8.1 ×10⁴ and 1.7×10⁴ conidia ml⁻¹ against *A. gossypii*, *B. brassicae* and *L. pseudobrassicae*, respectively. It also produced significantly higher conidia on cadavers compared to the other isolates, and was therefore selected for screenhouse experiments. In the screenhouse, aqueous and oil formulations of ICIPE 62 significantly reduced aphid population growth rate (r_t), *B. brassicae* -0.03 and -0.03 and *L. pseudobrassicae* -0.02 and -0.04 on kale, and *A. gossypii* -0.04 and -0.07 on okra, respectively; compared to the control (0.08 and 0.04 for *B. brassicae*, 0.01 and 0.01 for *L. pseudobrassicae*, and 0.03 and 0.01 for *A. gossypii*, respectively). These results are indicative of the potential of isolate ICIPE 62 in the management of aphids

Poster / Microbial Control. Wednesday, 16:30. **MC-2**

Virulence of fungal spores produced in liquid and solid state media on nymphs of *Trialeurodes vaporariorum*

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Quality of spores of five fungal entomopathogens, produced in liquid and on solid media, was assessed on nymphs of whitefly *Trialeurodes vaporariorum*. Isolates of *Lecanicillium attenuatum*, *L. muscarium*, *L. longisporum* and one unidentified *Isaria* sp. were first passed through larvae of *Tenebrio molitor* to enhance virulence. Three-times subcultured pure colonies were used to inoculate liquid or solid media to produce submerged and aerial spores. The liquid medium production system consisted of 250 mL Erlenmeyer flasks containing a mineral solution with a C/N ratio of 10/1 supplemented with yeast extract, placed in an orbital shaker at 180 rpm and 25°C. The solid medium production system consisted of Petri dishes containing PDA, placed in an incubator at 25°C. Spores were collected and suspensions of 1×10⁶ germinable spores were prepared. Five tomato leaves, infected with *T. vaporariorum* nymphs at 2nd-3rd instars, were submerged for one minute in the spore suspensions of each isolate, and maintained in 200 mL water-agar glasses in a growth chamber during ten days. The number of dead nymphs was evaluated six and ten days after inoculation. Control treatments consisted of ten leaves infected with the whitefly nymphs and treated with sterile water. Aerial spores of the *Lecanicillium* spp. isolates caused higher mortality than submerged spores. *L. longisporum* was the least affected by the production system. Contrary to *Lecanicillium*, submerged spores of the *Isaria* isolate killed more nymphs than aerial spores six days after inoculation. The production system should be considered during the screening and evaluation of microbial control agents.