

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)

Yuri Tokarev<sup>1</sup>, Vladimir Voronin<sup>2</sup>, Egor Rusakovich<sup>3</sup>, Irma Issi<sup>1</sup>

<sup>1</sup>All-Russian Institute of Plant Protection, St. Petersburg, Russia; <sup>2</sup>St. Petersburg Veterinary Medical Academy, St. Petersburg, Russia; <sup>3</sup>Herzen State Pedagogical University of Russia, St. Petersburg, Russia

Address for Correspondence: jumacro@yahoo.com

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*

Siva Kamalakannan<sup>1</sup>, Chandrakasan Gobinath<sup>2</sup>, Sivapunyam Ananth<sup>3</sup>, Kadarkarai Murugan<sup>1</sup>

<sup>1</sup>Division of Entomology, Department of Zoology, Bharathiar University, Coimbatore - 641 046, Tamil Nadu, India; <sup>2</sup>Bio control laboratory, Department of Biotechnology and Genetic Engineering, Bharathidasan University, Tiruchirappalli, Tamil Nadu, India; <sup>3</sup>Insect control division, Department of Biotechnology, Annai Arts and Science College, Kumbakonam, Tamil Nadu, India.

Address for correspondence: kamal410@yahoo.com

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

Dalia Muftah Alkhayat<sup>1</sup>, Katharina Döll, Petr Karlovsky, Stefan Vidal

Institute for Plant Protection and Plant Pathology, Georg-August University, Göttingen, Germany

Address for Correspondence:

d.muftahalkhayat@stud.uni-goettingen.de

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?

Vasiliki Gkounti<sup>1</sup>, Markogiannaki Dimitra<sup>2</sup>, Dimitris Kontodimas<sup>2</sup>

<sup>1</sup>SLU, Sweden, <sup>2</sup>Benaki Phytopathological Institute, Greece  
Address for Correspondence: vasiliki.gkounti@slu.se

The sisal weevil was first recorded in Greece on May 2010 on ornamental plants of *Agave sp.* As the use of synthetic insecticides is prohibited in urban landscape areas the evaluation of potential biological control agents (BCAs) is essential. Indigenous strains of *Isaria fumosorosea* and *Metarhizium anisopliae* isolated from the soil and a strain of *Beauveria bassiana* obtained from a *Rhynchophorus ferrugineus* cadaver were chosen for evaluation.

Infection of adults and larvae of *S. acupunctatus* was achieved by immersing individuals in aqueous conidial suspensions. Additionally, natural diet of insects was also immersed in conidial suspensions and provided to individuals, in order to assess effectivity of application through treated surface. *Beauveria bassiana* and *M. anisopliae* were applied in concentrations of  $10^7$  and  $10^6$  conidia/ml while *I. fumosorosea* was applied at a concentration of  $10^6$  conidia/ml. Mortality was recorded daily for up to 11 or 21 days for larvae and adults respectively. The highest adult mortality was achieved by *B. bassiana* through contact application reaching 100%, followed by *M. anisopliae* ( $48 \pm 10\%$  to  $28 \pm 10\%$ ) and *I. fumosorosea* ( $40 \pm 6.3\%$ ). In terms of larvae, mortality in all bioassays reached 100% with the exception of the treatment of contaminated diet by *I. fumosorosea* conidia ( $20 \pm 11\%$ ). All cadavers produced visible mycelium on their surface within a week. Results indicate a high level of mortality at the most harmful life stage of the pest, even at low concentrations and a lower level of mortality at the mobile adult stage. Benefits of a low concentration application of fungi are discussed.

Contributed paper. Wednesday, 15:00 **181**

**Understanding *Beauveria bassiana* infection within its host *Triatoma infestans*: time course expression of genes encoding fungal toxic nonribosomal peptides and insect humoral immune proteins**

Luciana S. Lobo<sup>1,2</sup>, Éverton K. K. Fernandes<sup>2</sup>, Christian Luz<sup>2</sup>, M. Patricia Juárez<sup>1</sup>, Nicolás Pedrini<sup>1</sup>

<sup>1</sup>Instituto de Investigaciones Bioquímicas de La Plata (CCT La Plata CONICET-UNLP), Facultad de Ciencias Médicas, Calles 60 y 120, 1900 La Plata, Argentina; <sup>2</sup>Instituto de Patología Tropical e Saúde Pública (IPTSP), Universidade Federal de Goiás, CP 131, 74001-970 Goiânia, Brazil  
Address for Correspondence: nicopedrini@yahoo.com

During invasion into insect hemocoel, some entomopathogenic fungi secrete toxins contributing to a successful infection. In response, insect cellular and humoral immune reactions are triggered. In this work, we studied by real-time PCR the expression pattern of *B. bassiana* genes involved in beauvericin, bassianolide, and tenellin biosynthesis throughout the infection process in nymphs of the kissing bug *T. infestans*. We also investigated the expression level of some bug proteins involved in the humoral immune response, i.e. prophenoloxidase, hemolectin and defensin. In conidia-treated insects, the expression of beauvericin synthetase, bassianolide synthetase, and tenellin synthetase peaked 6 days post-inoculation. In blastospore-injected bugs (bypassing the insect cuticle) the expression level peaked 12 hours post-injection. Regarding insect immune response, conidia treatment induced higher expression of defensin and hemolectin, with values of  $8.3 \pm 1.1$  and  $2.7 \pm 1.4$  fold inductions, respectively. In blastospore-treatment, the expression level of all genes tested raised from 12 to 48 hours, reaching  $9.3 \pm 3.6$  (prophenoloxidase) and  $26.6 \pm 5.4$  (defensin) fold induction. These results help to understand at the molecular level the "arm race" taking place in insect hemocoel during fungal invasion.

Contributed paper. Wednesday, 15:15 **182**

**Compatibility of herbicides used in olive orchards with a *Metarhizium brunneum* strain used for the control of the olive fly preimaginals in the soil**

Enrique Quesada-Moraga, Inmaculada Garrido-Jurado, Meelad Yousef

University of Córdoba, Department of Agricultural and Forestry Sciences, ETSIAM, 14071 Córdoba, Spain  
Address for Correspondence: cr2qumoe@uco.es

*In vitro* and *in vivo* studies were developed to evaluate the compatibility of the six most common herbicides applied to the soil of olive orchards with *Metarhizium brunneum* EAMa 01/58-Su strain against medfly *Ceratitis capitata* pre-imaginals. The fungus demonstrated high *in vitro* compatibility with the six active ingredients in malt agar medium, with growth rates (a) ranging between  $2.5 \text{ mm d}^{-1}$  and (Glyphosate) and  $3.3 \text{ mm d}^{-1}$  (Oxyfluorfen). This compatibility was also revealed *in vivo* by assaying the fungus towards medfly prepupating larvae in herbicide containing soil (at  $1.0 \times 10^8$  conidia g soil<sup>-1</sup>). Even if there was a decrease of the *M. brunneum* level until  $10^4$ - $10^5$  conidia ml<sup>-1</sup> in the soil 15 days after inoculation, mortality rates, which were in the range of 70-80%, did not differ significantly to the controls, except the ones observed in soils treated Glyphosate and its herbicide combinations, in which a significant 50% reduction of virulence was detected. These results reveal a general compatibility of *M. brunneum* with the most common herbicides applied to the soil of olive orchards, whereas a mixture of the fungus in the tank of the atomizer for a simultaneous treatment beneath the tree canopy is recommend for all active ingredients except Glyphosate.

Contributed paper. Wednesday, 15:30 **183**

**The Seed Corn Maggot and *Metarhizium* are Related to Maize Yield in an Organic, Cover Crop-Based Farming Systems Experiment**

Mary Barbercheck; Christina Mullen  
Department of Entomology, Penn State University, University Park, PA 16802 USA  
Address for Correspondence: meb34@psu.edu

Organic farmers must largely rely on cultural practices and biological processes to prevent crop damage from pests. Many farmers are interested in using cover crop mixtures to gain production and ecosystem benefits, but there has been little research on the effects of cover crop diversity on arthropod pests and their natural enemies. The seed corn maggot, *Delia platura* (Diptera: Anthomyiidae), is an early-season pest of large-seeded crops in conventionally tilled systems. Insect-pathogenic fungi in the genus *Metarhizium* commonly occur in agricultural soils and infect soil-dwelling arthropods. In 2013, we examined the effects of overwintering cover crop diversity, ranging from one to 7 species, on seed corn maggot fly emergence, *Metarhizium* detection, soil characteristics, and corn yield. Seed corn maggot was detected in post-plant emergence traps from all treatments in maize and soybean, with approximately 10 times greater numbers captured from maize compared to soybean. Numbers of flies captured were not related to level of cover crop diversity. *Metarhizium* was detected in all treatments, with similar average detection rates in maize and soybean. Detection of *Metarhizium* was not related to level of cover crop diversity. In multivariate analyses, numbers of emerged flies relates negatively to maize yield and detection of *Metarhizium*. *Metarhizium* detection relates positively to maize yield, soil organic matter, electrical conductivity, and Mg. The negative relationship between emergence of seed corn maggot flies and *Metarhizium* suggests that this fungus is a natural mortality factor for seed corn maggot at this site.