

The nematode *Deladenus siricidicola* Kamona, which sterilizes *Sirex noctilio* females, has been extensively and successfully used as a biological control agent for this woodwasp in the Southern Hemisphere. Curiously, a non-sterilizing (NS) strain of *D. siricidicola* is commonly found in North America and it is thought that the NS strain was introduced with *S. noctilio* when *S. noctilio* was introduced to North America. Finding an appropriate biological control agent in North America has been challenging due to the existence of native species of *Sirex* woodwasps that are not considered pests but are part of the decomposer community in forests. Therefore, evaluation of biological control agents requires studies of host specificity of the nematodes. For this experiment, we evaluated the NS strain of *D. siricidicola*, which is poorly understood and is a potential competitor of *D. siricidicola* Kamona. *D. siricidicola* has two forms: a form that parasitizes *S. noctilio* and a mycophagous form that feeds on the fungal symbiont of *S. noctilio*, *Amylostereum*. The goal of this study was to investigate associations between the NS nematodes and different isolates of the symbiotic fungus, mainly to evaluate the ability of the nematodes to develop and reproduce on different isolates of *Amylostereum* associated with *Sirex* in North America.

Poster / Nematodes. Wednesday, 16:30. **NE-16**

**Use of entomopathogenic nematodes in the biological control of gypsy moth *Lymantria dispar* (L.) (Lepidoptera: Lymantriidae)**

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The gypsy moth is one of the major insect pests, commonly distributed in west Georgia. Insect damages mainly foliage trees and spread easily from forest vegetation to fruit orchards. The aim of the research was to study efficacy of two species *S. carpocapsae* and local *S. thesami* against larvae of the gypsy moth in field conduction. Nematodes were reared produced in vivo, *Galleria mellonella* larvae. (Temperature=23°C and hygrometry=88-92%). Experiments against larvae of the gypsy moth were carried out in June, in the area adjacent to the deciduous forests of the Tbilisi National Park. Small, young crab-apple and wild pear trees were chosen for experiment. The average number of pest specimens on 1 m<sup>2</sup> branch of the each experimental plant was 74.3±4; 58.6±5; 85.2±6 and 78.3±5 on the control plant. About 30 liters of nematode suspension was used to treatment of experimental trees. One part of plants was treated with *S. carpocapsae* suspension 1500 IJs/ml of water, and the second part with the same dose of *S. thesami*. Experiments on the same pests were performed with increased concentration - 3000 IJs/ml of water. The calculation of the insect mortality in field conduction was carried out on the 7<sup>th</sup> day after treatment. The larval mortality rate was 77.5% - 63.3% where low concentration of nematodes was used. In the case of double concentration mortality was 88.6 and 76.3% respectively. On the basis of the results obtained it can be noted that *S. carpocapsae* proved to be more efficient (10-12%) compared with the local species *S. thesami*.

Poster / Nematodes. Wednesday, 16:30. **NE-17**

**The susceptibility of Colorado potato beetle *Leptinotarsa decemlineata*, and mulberry moth *Glyphodes pyloalis* to entomopathogenic nematodes, *Steinernema carpocapsae* and *Steinernema feltiae* in Georgia**

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Colorado potato beetle, *Leptinotarsa decemlineata* and mulberry moth, *Glyphodes pyloalis* are the major pest insects of vegetable and urban horticulture crops in Georgia. The aim of this study was to determine the efficacy of entomopathogenic nematodes *Steinernema carpocapsae* and *Steinernema feltiae* against *L. decemlineata* and *G. pyloalis* larvae under laboratory and field conditions. In the laboratory, *S. carpocapsae* and *S. feltiae* caused 92% and 62% larval mortality on *L. decemlineata*, respectively. *S. carpocapsae* also caused high mortality (74%) than *S. feltiae* (52%) in the field study. For *G. pyloalis*, *S. carpocapsae* induced greater larval mortality (82 and 72%) than *S. feltiae* (65 and 61%) under the laboratory and field conditions, respectively. In conclusion, *S. carpocapsae* exhibited significantly greater efficacy than *S. feltiae* against both insect species. The results suggest that *S. carpocapsae* has a great biological control potential against *L. decemlineata* and *G. pyloalis* larvae in Georgia. However, the efficacy of *S. carpocapsae* should be tested in large-scale field studies.

Poster / Nematodes. Wednesday, 16:30. **NE-18**

**Co-infection interactions between entomopathogenic fungi and *Steinernema feltiae* using *Tenebrio molitor* as a model system**

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Prior studies have been conducted investigating additive, synergistic, or antagonistic interactions between multiple types of biocontrol agents when co-infecting an insect host. Fewer studies have focused on combining entomopathogenic nematodes (EPNs) and entomopathogenic fungus (EF) to control weevils and scarab grubs. None of these studies have investigated interactions between *Steinernema feltiae* and EF. The present study investigates co-infection interactions between commercially produced *S. feltiae* and two isolates of EF, using *Tenebrio molitor* (Coleoptera) as a model host system. *T. molitor* larvae were infected with either *Beauveria* or *Metarhizium* isolated from naturally infected insects collected in strawberry fields in Denmark. At different intervals following EF infection, larvae were exposed to *S. feltiae*. The impact of fungal infection on the nematode was measured by counting the number of infective juveniles that penetrated the host in comparison to the number of infective juveniles that penetrated control larvae with no prior EF exposure. Daily mortality was recorded, and cadavers from nematode treatments were monitored for mycosis and placed on white traps in order to compare the total number of *S. feltiae* offspring produced in the presence of fungal infection. We discuss the use of *T. molitor* as a model system and the extrapolation of these results for the control of strawberry blossom weevil, *Anthonomus rubi*.

Poster / Nematodes. Wednesday, 16:30. **NE-19**

**Some observation on morphology and ecology of mollusc-parasitic nematode *Alloionema appendiculatum***

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*Alloionema appendiculatum* is a common larval parasite of many terrestrial molluscs. Its 3rd stage larvae (dauer juveniles) invade foot muscle of snails and slugs. Dauer juveniles

developed in to the 4th stage larvae, that leaves slugs. Later they mature and reproduce in the soil. Despite the fact this nematode is a parasite of snails in heliculture and also an invasive slug *Arion vulgaris* (syn. *A. lusitanicus*), that is one of the most serious pest in agriculture and horticulture, the knowledge about morphology and ecology of this nematode are very poor. We performed some studies of this nematode with a goal to provide new information about morphology, phylogeny and ecology of this species. This work brings, above all, the complete redescription of *A. appendiculatum*, include molecular biological characterisation suggesting high intraspecific variability in ITS region. Results of ecological studies provided new information about the saprobic life cycle and natural prevalence, but also show that, in standard conditions, *A. appendiculatum* has very weak influence on mortality and feeding activity of slugs *A. vulgaris*, while in other stressful conditions it might be an important agent controlling population density. But we concede that this can be also strongly influenced by bacterial associates, even though the role of bacteria in nematode development is questionable.

Poster / Nematodes. Wednesday, 16:30. **NE-20**

**Osmotic stress tolerance and infective juvenile production of entomopathogenic nematodes subject to fast host-desiccation treatments**

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Entomopathogenic nematodes (EPN) are being used commercially in several countries for the control of soil dwelling pests. However, their effectiveness is affected by environmental stresses such as low soil moisture. An alternate method for ensuring nematode's survival and infectivity is to apply them in the cadavers of *Galleria mellonella* used to reproduce them. It has been reported that the IJ's emerging from cadavers have increased infectivity and higher tolerance to low soil moisture and high temperatures. To determine the optimum time post infection and intensity of desiccation for higher IJ's production and their effects on osmotic stress tolerance in these EPN a laboratory experiment was carried out. Our results showed that timing to start desiccation (2, 4 and 6 days post-infection) and intensity (1, 2 and 4 days in a desiccator) affected weight reduction, especially in *S. glaseri*, which resulted in higher death rates of the IJ's. The total number of nematodes, however, was not related to the opportunity or intensity of the stress treatments, but to nematode species and initial weight of the hosts. In an evaluation of survivorship in a 30 % PEG-8000 solution, pre-conditioned *Heterorhabditis bacteriophora* showed a significantly higher tolerance to osmotic stress than *Steinernema glaseri* and showed an increase in tolerance 100 % larger than the observed with the last nematode species. The higher percent of survivorship was obtained with IJ's from hosts where desiccation treatments initiated 2 days post-infection in both EPN.

Poster / Nematodes. Wednesday, 16:30. **NE-21**

**Assessing entomopathogenic nematode population genetics: a research and teaching approach**

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While entomopathogenic nematodes (EPN) are important components of ecosystems, relatively little is known about the genetics of individual EPN populations in natural settings. We are combining an attempt to answer the question "How related are EPN found in natural settings?" with an integration of EPN into an undergraduate Genetics course module on population genetics. We used Random Amplified Polymorphic DNA (RAPD) approaches, and are working with lab maintained geographic isolates of EPN to identify appropriate primers and develop methodology. We have tested our technique by first assessing the genetic variability of a single geographic isolate of a single EPN species, and then exposed waxworms to a combination of geographic isolates of that species. We then assessed the genetic variability of the IJs that emerged from "mixed-isolate" waxworms. RAPD has been effective at identifying markers for individual geographic isolates, and for assessing the population genetics from "mixed-isolate" populations. RAPD is also a standard technique taught in Genetics labs, meaning that a high throughput of samples is possible and that undergraduates are exposed to real-world questions in the classroom. Once this technique has been fully developed for laboratory isolates, we plan to move this research effort into the local ('natural') environment, where we will answer the original question regarding the population genetics of local EPN isolates pre- and post-infection. This may improve our understanding of how natural populations are structured, and hopefully will provide insight that is relevant to the use of these organisms for biological control.

## VIRUSES

Poster / Viruses. Wednesday, 16:30. **VI-1**

**High-level Expression of Foreign Protein Using the Partial Polyhedrin-fused Baculovirus Expression System**

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Polyhedrin is the major component of the nuclear viral occlusions produced during replication of the baculovirus *Autographa californica* multicapsid nucleopolyhedrovirus (AcMNPV). To enhance the production efficiency of foreign protein in baculovirus expression system, the effects of various polyhedrin fragments were investigated by fusion expressing them with the enhanced green fluorescent protein (EGFP). Recombinant viruses were generated to express EGFP fused with polyhedrin fragments based on the previously reported minimal region for self-assembly and the KRKK nuclear localization signal (NLS). The marked increase of EGFP production by these fusion expressions was confirmed through protein and fluorescence intensity analyses. Among the fusion-expressed protein in nucleus and cytoplasm, the most hyper-expression was observed in the fusion of amino acids 19 to 110 and 32 to 59 of polyhedrin. The marked increase of production of several other foreign proteins was proved by the fusion expression with these polyhedrin fragments. This study suggests a new option for higher expression of useful foreign recombinant protein by fusion expression with the partial polyhedrin in baculovirus.