study of an atypical microsporidium infection in a feather mite (*Falculifer rostratus).* The infection is restricted to the *colon epithelium where it* leads to hypertrophy of the concerned cells. During sporogony multinucleate plasmodial aggregates are formed within a sporont. The sporonts are in direct contact to the host cell cytoplasm. Merogonial stages were not present. Spores are tiny (3.6 x 2.6 µm), broad ovoid in form and monokaryotic. The spore wall of mature spores has a thickness of about 240 nm and consists of a three-layered endospore and a thin, electron-dense exospore. The polar filament is anisofilar and arranged in 3–4 coils. In cross-sections it has a star-like appearance since the electron-dense core forms rounded compartments for lucent material at its surface. In grazing sections this results in a honeycomb-like pattern. A polaroplast is missing. The life cycle features and atypical spore structures clearly classify the species from the feather mite as a member of the order Chytridiopsida. Its affiliation to one of the known genera is discussed.

Poster / Microsporidia. Wednesday, 16:30. **MI-7**

Infectivity of a *Thelohania* like microsporidian isolated from *Phthonandria atrilineata* to the silkworm, *Bombyx mori*

Liangen Shi
College of Animal Sciences, Zhejiang University, Hangzhou, Zhejiang Province, China
Address for Correspondence: slgsilk@zju.edu.cn

The pebrine of the silkworm, *Bombyx mori*, is a disease caused by infection with the microsporidium *Nosema bombycis*, also can be caused by cross-contamination of microsporidium from wild insects. We have isolated a *Thelohania* like microsporidian (TMPA) from the *phthomndria atrilineata* in the silkworm rearing region of Zhjiang province, China. The mature spores of TMPA were cylindrical or ovocylindrical in shape with a strong diopter and glossy surface. The spore size of TMPA was 3.27±0.14×2.03±0.16 µm with a length/width ratio of 1.61±0.11 µm, similar to those of *N. bombycis*. Therefore, the spores of TMPA were hardly distinguished from the spores of *N. bombycis* under light microscope. In TMPA spores formative stages, sporont produced pansporoblast including 8 nuclears by meiosis, and later 8 spores were formed in pansporoblast. Infection was systemic with mature spores produced in muscular tissue, epithelial cell of trachea, fat body, middle and posterior silkgland, fore and middle intestine, malpighian tubule and germ gland, most extensivest in muscular tissue and epithelial cell of trachea, but not in dermal cells, nerve cells, fore silkgland, posterior intestine and hemocyte cells. The IC_{50} value of TMPA to newly-hatched silkworm larvae was 1.55×10^4 spores/ml, 700-fold higher than that of *N. bombycis*, suggesting a weakly infectiousness. TMPA have transovarian transmissibility in silkworm, the rate of transovarian transmission was 1.74%, which was significant lower than that of *N. bombycis*.

NEMATODES

Poster / Nematodes. Wednesday, 16:30. **NE-1**

First release of the mermithid *Strelkovimermis spiculatus* in *Culex pipiens* mosquito populations in Argentina

Evangelina Muttis[1]; María F. Achinelly[2]; María V. Micieli[3]
[1]Fellowship CONICET Centro de Estudios Parasitológicos y de Vectores, CEPAVE, calle 2 N◦ 584, 1900 La Plata, Argentina; [2,3]Researcher CONICET, Centro de Estudios Parasitológicos y de Vectores, CEPAVE, calle 2 N◦ 584, 1900 La Plata, Argentina
Address for Correspondence: fachinelly@cepave.edu.ar

Mermithids have proved to be effective in parasitizing natural populations of mosquito larvae. However nothing is known about the inoculative introduction of this nematode in natural populations of culicids in our country. We report the results of the first field release of *S. spiculatus* in Argentine. Study area was constituted by house drainage ditches, breeding site of the mosquito *Culex pipiens* where this nematode was not present. The number and stage of mosquitoes were recorded pretreatment. *Strelkovimermis spiculatus* was introduced as second-stage juveniles (J2) obtained from laboratory cultures maintained at CEPAVE laboratory. Release was done in November 2012 (spring). A dose of 10,000 J2 per meter was applied (over a total area of 17 x 0.5 m). The number of J2 was based on previous results. Mosquito larvae were sampled 24 hs post-treatment once a week during a year, to corroborate the presence of nematode by microscopic dissection and emergence from fourth instars larvae. Parasitism by *S. spiculatus* began to be observed at third day post-application (3%). Values ranged between 0.01% and 86.3%. The highest value was recorded at 8 months post-release. This environment remained dry or without larvae during a period of four months. Nevertheless a parasitism of 45.2% was observed after this period during the first larvae collection and reaching levels between 4.8% and 86.3%. Only in three occasions was not observed infected larvae throughout the year of sampling. *Strelkovimermis spiculatus* was able to establish itself in this habitat and cause high levels of infection in *Culex pipiens* larvae.

Poster / Nematodes. Wednesday, 16:30. **NE-2**

Increased infectivity in Steinernema websteri IJ after development in desiccation-stressed hosts

Andrea Binnebose and Susan M. Bornstein-Forst
Marian University, 45 S. National Ave., Fond du Lac, WI 54935 USA
Address for Correspondence: sbornsteinforst@marianuniversity.edu

This study investigates the effect of desiccation during development on entomopathogenic nematode (EPN) infectivity. *Galleria mellonella* hosts infected with *Steinernema websteri* A10 were allowed to air-desiccate in an environmental chamber set at 23^0C for up to 31 days post-infection (DPI) resulting in a host weight loss of approximately 64%. Host carcasses were re-hydrated using reverse-osmosis (RO) water and placed in White traps to collect emergent infective juvenile populations (IJ). IJ were pooled over a three-day time period for time points on days 10, 17, 24, and 31 DPI, respectively. For a randomly chosen sample of 100 IJ for each time point, sine wave movement (number of oscillatory motions completed in one minute) and IJ morphometrics, were measured. To evaluate IJ efficacy, plexiglass “bulls-eye” traps with screens dividing sections into quadrants of specific radii were loaded using sterile soil. Twenty hosts were placed in each quadrant in the outer ring only. A dose of 10,000 IJ from each time point was placed in the center ring. Host mortality was measured over 132 hour time period. Results demonstrated that IJ collected from desiccation-stressed hosts at days 17 and 24 post-infection were significantly smaller while exhibiting greater oscillation compared with controls ($\alpha \leq 0.5$). Furthermore, efficacy experiments using bulls-eye traps demonstrated that the same desiccation-stress IJ populations killed approximately 70% of hosts between 60-72 hours post load as compared 30% mortality between 72-84 hours post load for controls. This study has implications for host delivery systems in field applications.

Poster / Nematodes. Wednesday, 16:30. **NE-4-STU**

Characterization of symbiotic bacteria *Photorhabdus luminescens* subsp. *laumondii* associated with *Heterorhabditis bacteriophora* isolated from Turkey

Harun Çimen; Selçuk Hazır
Adnan Menderes University Faculty of Arts and Science Department of Biology, Turkey
Address for Correspondence: haruncimenn@gmail.com

The symbiotic bacteria of a novel entomopathogenic nematode *Heterorhabditis bacteriophora* isolate 48-02 was identified as *Photorhabdus luminescens* subsp. *laumondii*. This bacterial isolate did not exhibit typical signs of infection, e.g., red pigment and a gummy consistency in the host was lacking. *P. luminescens laumondii* strain 48-02 was more virulent in percentage mortality and time-to-kill compared with the molecularly similar *P. luminescens laumondii* TT01 strain. In specificity tests, *P. luminescens laumondii* strain 48-02 colonized in *H. bacteriophora* TT01 infective juvenile nematodes but the bacterial symbiont of TT01 did not colonized in *H. bacteriophora* 48-02 infective juveniles.

Poster / Nematodes. Wednesday, 16:30. **NE-5**

Pathogenicity of nematobacterial complexes and its development

Pavel Dobes; Jakub Berka; Jana Hurychova; Libor Vojtek; Pavel Hyrsl
Department of Animal Physiology and Immunology, Institute of Experimental Biology, Faculty of Science, Masaryk University, Kotlarska 2, 61137 Brno, Czech Republic
Address for Correspondence: pavel.dobes@mail.muni.cz

Entomopathogenic nematodes and their associated bacteria comprise together highly pathogenic complex able to invade and kill insect host within two days. Both bacteria and nematodes produce variety of factors interacting with insect immune system that help to overcome host defences. These factors are specific for each of nematobacterial complexes leading to the differences in their pathogenicity. Moreover, we observed difference in pathogenicity also between two isolates of one nematobacterial complex, *Steinernema carpocapsae – Xenorhabdus nematophila*. Ability to invade and kill insect host is low in newly emerged nematodes and develops through the time reaching its maximum after three weeks in complex *Heterorhabditis bacteriophora – Photorhabdus luminescens*. Differences in pathogenicity were observed also among particular generations of nematodes released from insect cadaver. Nematodes collected at the beginning of emergence were less pathogenic than subsequent collections. From third week of collection further we did not detect any other significant changes in nematobacterial pathogenicity, which is then influenced only by the survival of nematodes. Data describing development of infectivity and pathogenicity of *Heterorhabditis bacteriophora – Photorhabdus luminescens* complex will be used to increase efficiency and reproducibility of experimental infections used to describe immune response of insect to the nematobacterial complexes.

Our research was supported by the project KONTAKT II LH14047 and program CZ.1.07/2.3.00/30.009 co-financed from European Social Fund and the state budget of the Czech Republic.

Poster / Nematodes. Wednesday, 16:30. **NE-6**

Use of entomopathogenic nematodes to control vine weevils on Chilean berry orchards

Andrés France[1], Ernesto Cisternas[2], Irina Urtubia[1]
[1]Instituto de Investigaciones Agropecuarias (INIA), Quilamapu, Av. Vicente Méndez 515, Chillán, Chile, [2]INIA La Cruz, Chorrillos 86, La Cruz, Chile
Address for Correspondence: afrance@inia.cl

Vine weevils (Coleoptera: Curculionidae) are the most challenging pest in Chilean berry crops; they produce severe damage in the root system, decrease fruit yield and the longevity of the orchard. Besides, most of those species are quarantine pests, making obligatory their control to avoid fruit rejections in foreign markets. The control is difficult because larvae are deep into the soil or dwelling the main roots, avoiding pesticides or cultural practices. Entomopathogenic nematodes (EPN) are the most effective alternative to control these insects, because of their ability to search the larvae in the soil and even inside the dwellings. The Chilean collection of EPN (102 isolates) has been screened against the most important vine weevil affecting berries: Fuller's rose weevil *Asynonychus cervinus,* Grapevine weevil *Naupactus xanthographus*, Black vine weevil *Otiorhynchus sulcatus,* Plum weevil *Aegorhinus nodipenis* and Raspberry weevil *Aegorhinus superciliosus*. The most effective NEPs have been isolates of *Steinernema australe*, *S. feltiae* and *S. unicornum*. Average control is about 70% for these pests, measured through adult emergencies. Mass rearing has been accomplished by *in vivo* production in larvae of *Galleria mellonena* and *in vitro* through liquid media, with yields of 30-35,000 dauers/ml. NEP have been formulated in granules, gels and clays and storage up to 6 months, with 78, 80 and 72% of survivance and those dauers remain actives against the target insects. Field evaluations shows that NEP are an effective alternative for vine weevil control.

Poster / Nematodes. Wednesday, 16:30. **NE-7**

Nematodes of large larch bark beetle *Ips cembrae* (Coleoptera: Scolytinae)

Sarka Grucmanová[1]; Václav Čermák[2] , Jaroslav Holuša[1]
[1]Czech University of Life Sciences Prague; Czech Republic; [2]Central Institute for Supervising and Testing in Agriculture, Olomouc, Czech Republic
Address for Correspondence: grucmanova@fld.czu.cz

Nematodes of pest large larch bark beetle *Ips cembrae* were studied on three localities. Infestation by phoretic nematodes as well as infestation by endoparasitic nematodes in haemocel and intestinum was recorded. Phoretic nematodes were found under elytra, on wings or between body segments, especially between thorax and abdomen. It was the case of genus *Micoletzkya.* In haemocel adult females and juveniles of *Contortylenchus* sp., *Parasitylenchus* sp. and members of *Cryptaphelenchus* sp. were found. While in intestine the juveniles of *Parasitorhabditis* sp. and some tylenchid juveniles were found too. The large larch bark beetle gallery content was examined and adults of *Parasitorhabditis*, *Micoletzkya*, *Cryptaphelenchus*, *Bursaphelenchus* and *Laimaphelenchus* genera and some tylenchid juveniles were found.

This study was supported by Internal Grant Agency B0118/004 of Czech University of Life Sciences Prague.

Poster / Nematodes. Wednesday, 16:30. **NE-8**

Natural Occurence of Entomopathogenic Nematodes (Steinernematidae and Heterorhabditidae) in the Aydin district of Turkey

Baris Gulcu[1], Canan Hazir[2], Mehmet Karagoz[3], M. Alper Kesici[3]
[1]Düzce University, Faculty of Arts and Science, Department of Biology, 81620- Düzce, TURKEY; [2]Aydin Vocational School of Health Services, Adnan Menderes University, 09100 Aydin, Turkey; [3]Adnan Menderes University, Faculty of Agriculture, Department of Plant Protection, 09010 Aydin, Turkey
Address for correspondence: cananhazir@gmail.com

Entomopathogenic nematodes (EPNs) in the families Steinernematidae and Heterorhabditidae are lethal parasites of insects and used for biological control of soil insect pests. Because of favorable climatic conditions, the Aydin district is an important agricultural area which produces several exported valuable crops including strawberries (mostly in grown greenhouses), peaches, citrus, chestnuts, cherries and vegetables. Each product has specific or non-specific pests in the area and farmers have difficulties to overcome some of these pests with insecticides. The objective of this study was to determine the natural occurrence of entomopathogenic nematodes in the Aydin district of Turkey. A total 83 soil samples were collected between 2011-2012 to determine the diversity and distribution of EPNs. Nematodes were isolated using the insect baiting technique. Ten EPN isolates were recovered from 83 soil samples (8.3% positive). According to morphometric and molecular analyses (28S rDNA and ITS) six of the isolates were identified as *Heterorhabditis bacteriophora* Poinar, two isolates were *Steinernema feltiae* Filipjev and one isolate was *S. weiseri* Mracek, Sturhan & Reid.

Poster / Nematodes. Wednesday, 16:30. **NE-9**

Detection of dsRNA virus-like molecules in entomopathogenic nematodes

Noemi Herrero; Jiří Nermuť; Vladimír Půža; Zdeněk Mráček
Biology Centre of the Academy of Sciences of the Czech Republic, v.v.i. Institute of Entomology, České Budějovice, Czech Republic
Address for Correspondence: noemihas@gmail.com

Viruses have been largely viewed as pathogens; nonetheless, even if most of the studied viruses are detrimental to their hosts, some of them have been reported to be also beneficial or symptomless. They are ubiquitous, and have been described infecting almost all types of organisms, from other viruses and bacteria, to animals, plants, fungi, or even protozoa. Surprisingly, little is known about viruses which naturally infect nematodes, even if they are among the most abundant animals on Earth. Nevertheless, RNA viruses infecting *Caenorhabditis* species and the soybean cyst nematode have been recently detected thanks to next generation sequencing (NGS) technologies.
Many viruses associated with persistent and symptomless infections are known to have dsRNA genomes. The presence of dsRNA molecules of sizes ranging from 1 to 14 kbp have been used as indicator of virus infection in plants and fungi. This nucleic acid can represent genomes of dsRNA viruses, as well as replicative forms of viruses with ssRNA genomes. According to this, the main objective of this work was the discovery of new viruses among a collection of entomopathogenic nematodes by using dsRNA virus-like molecules detection, which constitutes a cheaper and faster technic if compering with NGS technologies. At the present time a total of 27 strains belonging to 12 different nematode species were analyzed. Two dsRNA virus-like molecules of approximately 2.4 and 2.3 kbp were detected infecting one of the analyzed species, *Steinernema huense*. These molecules could correspond to the genome of the first identified virus infecting an entomopathogenic nematode.

Poster / Nematodes. Wednesday, 16:30. **NE-10**

Celullar and humoral interactions between the white grub, *Polyphylla adspersa* Motschulsky (Col., Melolonthidae) and entomopathogenic nematodes

Jamileh Avandi[1], Javad Karimi[1], Mohammad Ghadamyari[2] & Ahmad Asoode[3]
[1]Biocontrol and Insect Pathology Laboratory, Department of Plant Protection, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran
[2] Department of Plant Protection, College of Agriculture Science, University of Guilan, Rasht, Iran
[3]Department of Chemistry, Faculty of Science, Ferdowsi University of Mashhad, Iran
Address for correspondence: jkb@ferdowsi.um.ac.ir

The interaction between the white grub larvae, *Polyphylla adspersa* Motschulsky (Col., Melolonthidae) and entomopathogenic nematodes (EPN), *Heterorhabditis bacteriophora* and *Steinernema gaseri* was addressed here. Differential Hemocyte Count (DHC) for the both second and third instar larvae of the white grub, granulocytes (65.25± %2.22) and plasmatocytes (22.14± %1.14) were most abundant cell types in the circulating hemolymph. Study on hemocyte and humoral reactions of the white grub larvae against the EPNs was performed by injection 20 monoxenic infective juveniles (IJs) of *S.glaseri* and *H. bacteriophora* into the insect hemocoel. The hemocel of the larvae at different hours post injection (hpi) was dissected and showed changes in total hemocyte count (THC), DHC and cell shape. Encapsulation was a typical cellular reaction, which its maximum rate was observed by 8 hpi of *H. bacteriophora* and 12 hpi of *S. glaseri.* The encapsulation reaction in third instar larvae was observed stronger than those of the second instar larvae. Also the encapsulation reaction against the *H. bacteriophora* had significant different with those against *S. glaseri*. In contrast to second instar larvae, third larval stage had higher specific phenloxidase activity when challenged with both EPNs species. It was showed the defense system could create initial melanization at 18 hpi of *S. glaseri* and 12 hpi of *H. bacteriophora.* However, EPNs probably reduced the hemocyte number in circulating hemolymph by their symbiotic bacteria. This occurrence which followed by reduce in THC level decreased the cellular and humoral intensity response of the larvae. Therefore, the immune system of the grub was suppressed by the EPNs while this system was activated in early stage of infection. This study showed weak immunity response of the white grub larvae of *P. adspersa* against EPNs, *S. glaseri* and *H. bacteriophora*. This finding could be helpful for the pest management by select the suitable EPN species in term of virulnce and ability to suppress the insect defense system.

Poster / Nematodes. Wednesday, 16:30. **NE-11**

***Oscheius rugaolensis*, new genus and species of insect parasitic nematodes from Iran**

Reyhaneh Darsouei & Javad Karimi
Biocontrol and Insect Pathology Laboratory, Department of Plant Protection, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran
Address for Correspondence: jkb@ferdowsi.um.ac.ir

During 2013, a survey was carried out to determine the pathogens of *Polyphylla adspersa* (Col; Scarabaeidae) in the Mashhad region, North East of Iran. All larval instars of *P. adspersa* with sign of nematode infection were collected and death larvae were transferred to the White trap. By using this method fifteen nematode isolates were isolated. Among the pathogenic agents, entomophatogenic and parasite nematodes had a moderate frequency. The initial identification of the collected nematodes carried out using morphometric data. Subsequently, molecular identification and phylogenetic analysis were performed using DNA sequences of ITS and 18SrDNA genes. The molecular data indicated that wg10 and wg19 isolates belong to *Oscheius* genus with 99% bootstraps support. Also, Nblast analysis introduced two isolates wg10 and wg19 as *O. rugaolensis*. The sequence of 18S gene *O.*

rugaolensis differed with wg10 and wg19 in 8 and 1 nucleotides, respectively. While on the basis of ITS sequences, 7 nucleotides were differed. The phylogenetic relationship was analysed based on bayesian procedure. In the reconstructed phylogenetic tree, wg10 and wg19 isolates were placed together with *O. rugaolensis* in a clade by 100% bootstraps support. The phylogenetic results from both genes, ITS and 18S, were similar. This is the first report of *Oscheius* genus for Iran. Despite the free living behavior of this species, it had high virulence on some insect species and higher ability to reproduce on the cadavers of *Galleria melonella* rather than healthy larvae. Future studies may provide more data about ability of this species as biocontrol agent.

Poster / Nematodes. Wednesday, 16:30. **NE-12**

Reproduction status of *Tribolium castaneum* affects its response to infection by *Steinernema feltiae*

Dariusz Małek[1], Joanna Homa[2], Maria Gaweł[1], Paulina Kramarz[1]
[1]Institute of Environmental Sciences, Jagiellonian University, 30-387 Krakow, Poland
[2]Institute of Zoology, Jagiellonian University, 30-387 Krakow, Poland
Address for Correspondence: paulina.kramarz@uj.edu.pl

Gender specific reproductive roles are a reason of sexual dimorphism not only in a body size but also in a whole range of physiological traits. We investigated differences between sexes as well as reproduction status (virgin vs. reproducing) of the red flour beetle, *Tribolium castaneum* in defence against infection by the nematode, *Steinernema feltiae*. Females and males of the beetles either virgin or after copulation were exposed individually to the nematodes. The beetles during infection were kept without food. From each group 20 individuals were sampled after 12, 24, 36 and 48 hours. Ten individuals of each sample were dissected and checked for the presence of the nematodes, ten were frozen for further phenoloxidase activity measurements.
Reproduction strongly affected the response of females – they mortality and parasite load was the highest among all studied group. This group had also the lowest phenoloxidase activity. At the same time, we did not observed differences between virgin beetles as well as between virgin and reproducing males. Surprisingly, eggs production itself did not increase females vulnerability to parasite – we observed eggs also in the body cavity of virgin females. Probably production of unfertilized eggs is less expensive than fertilized ones. The highest parasite load we found just after infection and after 48 hours. Last outcome can be explained by starvation of the beetles so they were weakened and the nematodes more easily infected them. Our results confirm that cost of reproduction may impair defence mechanism and immunological system of *T. castaneum* females..

Poster / Nematodes. Wednesday, 16:30. **NE-13**

Effect of culture type, container type, and temperature on a Korean strain of the entomopathogenic nematode, *Steinernema carpocapsae*

DongWoon Lee[1]; Ho Yul Choo[2]
[1]Major of Applied Biology, School of Ecological Environment and Tourism, Kyungpook National University, Sangju, Republick of Korea; [2]Department of Applied Biology, College & Institute of Agriculture & Life Sciences, Gyeongsang national University, Jinju, Republick of Korea
Address for Correspondence: whitegrub@knu.ac.kr

A Korean isolate of the entomopathogenic nematode (EPN) *Steinernema carpocapsae* KCTC 0981BP strain (ScK) is effective for control of many Korean agricultural and forestry pests. In vitro culture is available for large scale of mass production of commercial EPN, but it is a costly and complicated process, whereas in vivo culture using great wax moth *Galleria mellonella* larvae is simpler for small scale production. However, culture type and storage temperature during in vivo culture may influence harvesting and survival of EPN. We investigated effects of those factors on harvest, survival, and pathogenicity of ScK. Storage period, culture method, and storage container and temperature all influenced ScK survival. ScK survived better in small cultures rather than in mass culture, and better in Zip-lock containers than in tissue culture container. The best storage temperatures for ScK were 13 and 20℃ in small scale culture while there were no differences among temperatures in mass culture. The highest yields of ScK were obtained by rearing them in small cultures and keeping them in Zip-lock containers at 20 or 13℃. The pathogenicity of ScK differed among treatment combinations on the 1st day after inoculation, but there were no differences on the 3rd day. The number of established nematodes differed depending on storage temperature and period.

Poster / Nematodes. Wednesday, 16:30. **NE-14**

Steinernema feltiae (Nematoda: Steinernematidae) to control fungus gnat, _Bradysia mabiusi_ (Diptera: Sciaridae): effect of dosage and application time*

Patricia Ballone[1]; Luis G. Leite[1]; Fabio S. Schmidt[2]; Victória R. Campos[1]; Roselaine N. S. Bueno[1]
[1]Instituto Biológico, CEIB, CP70, Campinas, SP 13001-970, Brazil, [2]Bio Controle, Rua Ema Gazzi Magnusson, 405, Indaiatuba, SP 13347-630, Brazil. *Supported by FINEP, FAPESP, CNPq, and Bio Controle Company
Address for Correspondence: lgleite@biologico.sp.gov.br

Fungus gnat, *Bradysia* spp., is a pest of worldwide importance for nursery plants. The larvae feed on the roots of emerging plants, becoming potential target to use of entomopathogenic nematodes. This study aimed to determine the best time for the application of *Steinernema feltiae* after the exposition of the substrate (inside the pot) to the insect adults. Sixteen treatments were considered: the nematode applied at three doses 3, 14 and 70 IJs/cm^2 (173, 883 and 4417 IJs/pot, respectively), applied soon after the infestation of the substrate with adults, as well as by 7 days, 14 and 21 days after, plus the respective controls. For each treatment, four replications were considered, with each replication composed by a plastic pot (200 ml) containing 50 g of substrate (10% humidity) and 3 grains of black bean (pre-cooked) gathered on the substrate surface, on the center of the pot, for larval feeding. The pots were transferred to inside of a large cage containing the insect rearing and exposed to the adult population for 2 hours to allow insect oviposition. Then, the pots were transferred individually to inside of other chambers (1 liter) containing a double yellow plastic sheets (8.0cm x 8.0cm) covered with insect glue for attracting and capture the emerging adults. The best time for application of the nematode was 3 weeks after the exposition of the substrate to adults, providing 61, 69 and 78% control for the doses of 3, 14 and 70 IJs/cm^2.

Poster / Nematodes. Wednesday, 16:30. **NE-15**

The non-sterilizing strain of *Deladenus siricidicola* (Tylenchida: Neotylenchidae) and its development on different strains of *Amylostereum* (Basidiomycota: Russulales)

Isis A. L. Caetano, Ann E. Hajek
Department of Entomology, Cornell University, Ithaca, New York, United States
Address for correspondence: ial26@cornell.edu

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)

Manana Lortkipanidze, Oleg Gorgadze, Madona Kuchava, Nana Gratiashvili, Mzia Kokhia, Nino Gabroshvili
Institute of Zoology, Ilia State University, Tbilisi, Georgi
Address for Correspondence: tami@dsl.ge

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=23ºC 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^2 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^{th} 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

Nona Mikaia
Sokhumi State University, Tbilisi, Georgia
Address for Correspondence:nonamikaia@gmail.com

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

E. Erin Morris, Annette B. Jensen, Anja A. Wynns, Jørgen Eilenberg
Department of Plant and Environmental Sciences, University of Copenhagen, Frederiksberg 1871, Denmark.
Address for correspondence: morris@plen.ku.dk

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*

Jiří Nermuť, Vladimír Půža, Zdeněk Mráček
Biology Centre ASCR v.v.i., Institute of Entomology, Branišovská 1160/31, 370 05 České Budějovice, Czech Republic
Address for Correspondence: nermut@entu.cas.cz

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 juvenilles

develope 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 helicicultre 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 variabily 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 week influence on mortality and feeding activity of slugs *A. vulgaris*, while in other stressful conditions it might be an important agent controling 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

Jaime Ruiz-Vega[1], Teodulfo Aquino-Bolaños[1], Juan R. Delgado-Gamboa[2] and Carlos I. Cortés-Martínez[2]
Becarios [1]COFAA-IPN y [2]PIFI-IPN
Laboratory of Biological Control, CIIDIR U. OAXACA, IPN; Calle Hornos 1003, Santa Cruz Xoxocotlan, Oax., México, 71230
Address for correspondence: jvega@ipn.mx

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

Abigail Lewis, Logan Jefferson, Glen Stevens, Michaela Gazdik
School of Natural Sciences and Mathematics, Ferrum College, Ferrum, VA, USA
Address for Correspondence: gstevens3@ferrum.edu

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

Sung Min Bae[1]; Tae Young Shin[1]; Jae Bang Choi[1]; Yeon Ho Je[2]; Byung Rae Jin[3]; Soo Dong Woo[1]
[1]Department of Agricultural Biology, Chungbuk National University, Chungju, Korea; [2]Research Institute for Agriculture and Life Sciences, Seoul National University, Seoul, Korea; [3]College of Natural Resources and Life Science, Dong-A University, Busan, Korea
Address for Correspondence: sdwoo@cbnu.ac.kr

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.