

NEMATODES 1Contributed paper. Monday, 16:30. **35****Measuring entomopathogenic nematode activity, abundance and soil food web assemblage in Swiss wheat and maize cultivation**Raquel Campos-Herrera¹, Geoffrey Jaffuel¹, Xavier Chiriboga¹, Rubén Blanco-Pérez¹, Marie Fesselet², Vladimír Půža³, Fabio Mascher², Ted C.J. Turlings¹¹FARCE Laboratory, University of Neuchâtel, Emile-Argand 11, Neuchâtel CH 2000 (Switzerland); ²Département fédéral de l'économie, de la formation et de la recherche DEFR,

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Wheat and maize are major crops in Switzerland. As part of a research consortium that explores ways to improve soil health, we study how entomopathogenic nematodes (EPNs) can be better exploited for the biological control of soil-dwelling insect pests. We evaluated the impact of different agricultural management practices on native EPN populations in two 30-years Swiss field trials. One experiment compared tillage versus no-tillage and monoculture (wheat) versus crop rotation (maize), whereas the second studied four levels of tillage in two soil types planted with wheat. Soil samples were taken in April and in October 2013 (n = 88). Total nematode activity, as recorded with the *Galleria*-bait technique was <5%, with no significant effect of the treatments. Real time qPCR revealed that >95% of infected cadaver contained a mix of EPN with the competing *Acrobeloides* -group and/or *Oscheius* sp. The available molecular probes identified and quantified 13 organisms from soil, comprising six nematophagous fungi (NF), ectoparasitic bacterium, two free-living nematodes (FLN), and four EPNs (the evaluation of an additional ten EPN species is ongoing). In general, only trace levels of EPN were detected in all soils. *Heterorhabditis* spp. were the dominant EPN, with *H. bacteriophora* being significantly reduced by tillage ($P < 0.001$). Monoculture favored the competitors of EPN ($P < 0.01$). The abundance of EPN, NF and FLN was positively correlated ($P < 0.05$). Since only low numbers of EPN are naturally present in Swiss agricultural soils, an augmentation strategy may help to improve the control of root pests of wheat and maize.

Contributed paper. Monday, 16:45. **36-STU****Biocontrol and nutrition: understanding the role of environment in the trait deterioration of an entomopathogenic nematode symbiont**Dana Blackburn, Burke Crawford, and Byron Adams
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Entomopathogenic nematodes (EPNs; genera *Heterorhabditis* and *Steinernema*) kill their invertebrate hosts with the aid of a mutualistic bacterium. The bacteria (*Xenorhabdus* spp. for steinernematids and *Photorhabdus* spp. for heterorhabditids) are primarily responsible for killing the host and providing the nematodes with nutrition and defense against secondary invaders. EPNs are amenable to laboratory rearing and mass production for biocontrol applications against insects; however,

EPNs and their symbiotic bacteria exhibit trait deterioration or changes due to laboratory rearing. The overall goal of this project is to understand how virulence in the nematode-symbiont *Photorhabdus* has evolved in an *in vitro* environment and the role nutrition plays in this process. Nutritional effects in trait deterioration were determined using monoxenic cultures of a freshly isolated strain of *P. luminescens* subsp. *luminescens* where base populations were compared with bacteria that were sub-cultured repeatedly to determine fitness loss. Trait stability was monitored in three different liquid media that are frequently used in laboratory culture: Liquid Lipid Medium, nutrient broth, and tryptic soy both + 0.5% yeast extract. Subpopulations were compared to base populations for inclusion bodies after 5, 15, and 20 liquid growth cycles (of 48 hours each). Additionally, growth curves and LT_{50} values were determined for the base and deteriorated populations. Differences were observed among the media types and the base/deteriorated populations. Understanding nutritional effects on important biocontrol traits may aid in more efficient methods of mass production.

Contributed paper. Monday, 17:00. **37****Insect-killing nematodes also kill competitors: lethal male-male fighting in *Steinernema***

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Steinernema spp are well-known as entomopathogenic nematodes. We have found that males of certain species fight and kill each other, and that killing is influenced by the developmental pathway followed. The transmission stage of *Steinernema* is an infective juvenile (IJ) analogous to the dauer juvenile of *Caenorhabditis elegans*. IJs seek out and enter living insects in soil. Inside the insect they release symbiotic bacteria (*Xenorhabdus* spp.) which proliferate and digest the host tissues. This provides a rich nutritive medium for the developing nematodes, which reproduce in the host cadaver. A large host may support several generations of nematodes, and thus represents a valuable resource, worth competing for. In *Steinernema longicaudum*, males of the founding generation (those developing from IJ) fight by wrapping their tail ends around each others' bodies and squeezing. Victims may appear paralysed within minutes of such an encounter, and frequently die. Worms that develop within the host cadaver in second or later generations develop directly, without passing through the IJ stage. For such worms, the benefits of fighting (the quality of the resource) is diminishing, while the large number of rivals present means that the benefits of killing do not necessarily accrue to males that kill. We have found that males that develop directly, without passage through the IJ stage, are much less likely to fight than those that do, and that this appears to be a developmental effect rather than a response to conditions at the time of fighting.

Contributed paper. Monday, 17:15. **38-STU****Comparison of Life History Traits of the Entomopathogenic Nematodes *Steinernema feltiae* and *Steinernema riobrave***Temesgen Addis^{1,3}, Asmamaw Teshome², Olaf Strauch³ and Ralf-Udo Ehlers³¹Faculty of Agricultural and Nutritional Sciences, Christian-Albrechts-University, Hermann-Rodewald-Str.4, 24118 Kiel, Germany, ²Department of Biology, Ghent University, K.L. Ledeganckstraat 35, B-9000 Ghent, Belgium,

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Life history traits (LHTs) of *Steinernema feltiae* and *S. riobrave* were assessed at 25°C using a hanging drop technique. The LHTs were studied with 5 x, 10x and 20x 10⁹ cells ml⁻¹ of *Xenorhabdus bovienii* and *X. cabanillasii* for *S. feltiae* and *S. riobrave*, respectively, in semi-fluid nematode growth gelrite. The results indicated that increased food density had a significant positive influence on offspring production and net reproductive rate (R_0) on both, *S. feltiae* and *S. riobrave*. Highest offspring production was recorded at bacterial food densities of 20 x10⁹ cells ml⁻¹ with 813/female for *S. feltiae* and 1,913 offspring/female for *S. riobrave*. Higher R_0 values of 707 and 1,903 were recorded for *S. feltiae* and *S. riobrave*, respectively. A significant positive correlation between bacterial density and body volume that contributed to an increased offspring production was found in both species. The lowest intrinsic rate of natural increase (r_m) (1.1 days) was recorded for *S. feltiae* and the highest (1.4) for *S. riobrave*. A population doubling time of PDT = 0.6 days was recorded for *S. feltiae* and 0.5 days for *S. riobrave*. The life span of female nematodes was not significantly different among the bacterial food densities tested. Significant differences in offspring production and population growth rate were recorded between the two species. The result can be used to further investigate the optimal bacterial food density for mass production in bioreactors for maximum DJ recovery in liquid bacterial suspension, synchronised population development and DJ yields of *S. feltiae* and *riobrave*.

Contributed paper. Monday, 17:30. **39 STU**

How does plant domestication influence entomopathogenic nematodes as potential biological control agents?

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We are studying the effects of plant domestication on belowground plant induced responses to herbivore feeding and how this affects entomopathogenic nematode (EPN) populations. In the New Jersey Pinelands, natural populations of highbush blueberries (*Vaccinium corymbosum*) are commonly found adjacent to commercial fields of domesticated highbush blueberries. In a 3-year field study, we found that EPN were more prevalent but less diverse in cultivated fields than in natural stands. The dominant species in both habitats was *Steinernema glaseri* (Sg): numerous isolates of two distinct Sg strains were identified. In laboratory studies with oriental beetle (*Anomala orientalis*) larvae, the dominant root-feeder in cultivated fields, Sg blueberry isolates were less virulent than the Sg NC strain, and strains from cultivated fields tended to be more virulent than those from natural stands. We are using the same Sg strains in laboratory and field studies on EPN attraction to blueberry roots as affected by oriental beetle feeding. Ongoing studies suggest that Sg is attracted more strongly by damaged roots. We have yet to identify any herbivore induced plant volatile (HIPV) responsible for enhanced attraction. A comparison of 2 known HIPVs emitted from roots in other systems suggests that (E)- β -caryophyllene is more attractive than pregeijerene.

Contributed paper. Monday, 17:15. **40**

Analysis of intraspecific variability in *Steinernema kraussei* populations using PCA

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Species determination in Entomopathogenic Nematodes of the genera *Steinernema* and *Heterorhabditis* is a very complex task, given the broad variability of both morphological and morphometric traits within a single species. To accomplish that, molecular techniques have been adopted which however require additional knowledge. Particularly relevant would be the possibility of testing in a reliable way the variability between different populations of the same species, which might represent different strains with different biological properties. Aim of our work was to determine if morphometric analysis, performed using the "Principal Component Analysis" approach, was able to get evidences of characters with significant diagnostic value, allowing to make reliable distinctions among strains. Four strains of *Steinernema kraussei* were found in Italy, three from Sicily and one from Alps (Tarasco *et al.*, 2014;doi:10.1017/S0022149X14000194). Morphometric analysis of morphological traits commonly used in nematode taxonomy (referred to as *variables*) was done on 20 juveniles, males and females of first and second generations (or *observations*) belonging to three strains: 3D and PL (Sicily) and BT (Alps). Statistics was done by SIMCA package v.13. Up to three components were routinely computed; score plots, loading plots, X/Y overview and contribution plots were obtained. Our results showed that some of the morphometric variables employed could reliably be used to discriminate both juvenile and adult forms of PL strain, whereas an insufficient distinction could be made between BT and 3D.

Contributed paper. Monday, 17:30. **41**

Population genetic structure of entomopathogenic nematode *Steinernema affine* (Steinernematidae: Nematoda) inferred using microsatellite markers

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Population genetic structure of entomopathogenic nematodes is still poorly understood even though such knowledge could help us to assess stability and vulnerability of natural EPN populations. Molecular markers used in EPN taxonomy and phylogeny (ITS and LSU regions of rDNA, NAD4, COII) are too conservative to be used to assess within species variability. So far only few studies attempted to use AFLP method to investigate EPN intra-population variability. In present study, microsatellite markers for *Steinernema affine* were developed. In total 218 bioinformatically validated pairs of primers for various oligonucleotides were obtained. Thirty most promising oligonucleotides were selected and tested for the use in the study of the species' population genetic structure. Markers showing variability were identified and examined in various populations of *S. affine*, collected mainly in the area of South Bohemia..

Contributed paper. Monday, 17:45. **42 STU**

Eat or Be Eaten: Fungus and Nematode Switch off as Predator and Prey

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The parasitic nematode *Deladenus siricidicola* is widely used for the biological control of the invasive pine-killing woodwasp, *Sirex noctilio*. The nematode has a unique life cycle where it lives in pine trees, feeding on the symbiotic fungus of *S. noctilio*, the basidiomycete white rot fungus *Amylostereum areolatum*. In the presence of *S. noctilio* larvae, however, the nematode develops into a parasitic form which invades the woodwasp larvae, ultimately leading to sterilization of the host. The fungal-feeding stage of the nematode is used to commercially mass produce it for biological control programs. Previous studies investigating the effect of *A. areolatum* strain on *D. siricidicola* reproduction suggested the possibility of a role reversal where the fungus could eat the nematode. The present study examined the relationships between three species of *Deladenus* nematodes and their associated *Amylostereum* fungi. For *D. siricidicola* and *A. areolatum*, we hypothesized that significantly fewer nematode eggs placed in petri dishes containing potato dextrose agar medium would hatch in the presence of *A. areolatum* fungus than in control petri dishes with no fungus. Results supported this hypothesis. Additionally, light microscopy, fluorescence microscopy, and cryogenic scanning electron microscopy were used to show the ability of both *A. areolatum* and a second species, *A. chailletii*, to penetrate nematode eggs and adult living females of three species of *Deladenus* nematodes.

CONTRIBUTED PAPERS Monday, 16:30-18:30

VIRUSES 2

Contributed paper. Monday, 16:30. **43**

Insect feeding induces transgenerational resistance to NPV in Lepidoptera

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When population density increases, insects experience a number of stresses as a result of crowding including alteration in food quality and quantity. These changes have been shown to alter the resistance of insects to pathogens. Previous studies have tended to investigate the impact of these factors individually: however, in nature density-related factors are likely to interact simultaneously. While changes that occur within a single generation have been well documented, we know less about the transgenerational impact of changes in food quality on disease resistance. We tested the impact and interaction of three factors that are likely to occur in insect populations when density rises using the western tent caterpillar, *Malacosoma californicum pluviale*. Western tent caterpillars exhibit population cycles every 8-11 years, which

are characterized by NPV epizootics at high density. We manipulated food quality, food quantity and the presence of phylloplane bacteria in the parental generation and measured the impact on immunity and resistance to NPV in the offspring. The treatments, particularly the foliar treatments, had clear impacts on the disease resistance of the offspring generation: however, not necessarily in the direction predicted. We discuss these data in relation to how changing levels of susceptibility could influence population cycles in these forest insects.

Contributed paper. Monday, 16:45. **44**

The resistance of *Cydia pomonella* against baculoviruses is provoked by a mutation of the immediate-early *pe38* gene of *Cydia pomonella* granulovirus

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The *Cydia pomonella* granulovirus (CpGV) (*Baculoviridae*, genus *Betabaculovirus*) is a worldwide used biological agent to control the infestation of pome fruits by codling moth (*C. pomonella* L.). In 2005, first CM field populations resistant to commercial CpGV products containing the isolate CpGV-M (so-called Mexican isolate) were discovered in Europe. These resistant CM populations showed 1,000 – 100,000fold reduced susceptibility to CpGV-M when compared to normally susceptible CM populations. Infection experiments with isolates from different geographical origins showed that various CpGV isolates were able to overcome CM resistance in the genetically homogenous resistant laboratory CM strain. Molecular analysis of these resistance overcoming isolates (-I12, -I07, -S, and -E2) showed that the only genomic difference, which all resistance overcoming isolates have in common, is a single common 24 nucleotide indel mutation coding for eight amino acids within the immediate-early gene *pe38*. Phylogenetic analyses presume that this mutation is an insertion within the genome of CpGV-M.

Therefore, the role of *pe38* in overcoming the resistance of CM was analyzed by constructing knockout and rescue pseudoviral mutants based on a CpGV-M bacmid. According to the source of *pe38*, we could show that the pseudoviruses are infective against susceptible larvae only - in the case of *pe38* from CpGV-M - or against both susceptible and resistant larvae - in the case of *pe38* from CpGV-S. Therefore, we conclude that *pe38* is not only an essential factor for the infectivity of CpGV but also the key factor in overcoming CpGV resistance in CM.

Contributed paper. Monday, 17:00. **45**

CpGV-R5 allows replication of CpGV-M in resistant host insect larvae

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In resistant codling moth larvae, CpGV-M replication is blocked at an early step in all tissues. Among others, the CpGV-R5 isolate is able to overcome this resistance. A genetically heterogeneous virus population, containing 1% CpGV-R5, and