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## Abstracts: Alphabetically by first author.

OCCURRENCE AND DAMAGE OF THE BLOAT NEMATODE TO GARLIC IN NEW YORK. **Abawi[1], George S., K. Moktan[1], C. Stewart[2], C. Hoepting[3], and R. Hadad[4].** [1]Department of Plant Pathology and Plant-Microbe Biology, NYSAES, Cornell University, Geneva, NY 14456; [2]CCE, Troy, NY 12180; [3]CCE, Albion, NY 14411; [4]CCE, Lockport, NY 14094.

The stem and bulb (bloat) nematode (*Ditylenchus dipsaci*) occurs in numerous biological races and many of them are known to be highly destructive plant-parasitic nematodes of several crops, including garlic and onion. The first report of the bloat nematode in the USA infecting onion was from Canastota, NY in 1929 (reported in 1931) and on garlic from San Juan, California in 1931 (reported in 1935). Severe symptoms of damage to garlic by the bloat nematode were first observed in a field in western NY in June 2010, with as high as 80-90% crop loss in sections of the field. Severely infected garlic plants exhibit stunting, yellowing and collapse of leaves and premature defoliation. The bulbs of infected plants initially show light discoloration, but later the entire bulb or individual cloves become dark brown in color, shrunken, soft, light in weight, with a deformed basal plate and eventually exhibit cracks and various decay symptoms due to the additional activities of numerous saprophytic soil organisms. Severely infected bulbs are culled out when visible, as they are unmarketable. As a result, a statewide survey was conducted to assess the distribution of this nematode throughout the garlic producing areas in the state. A total of 88 symptomatic garlic samples were collected from garlic producers throughout the state and analyzed for the presence of the bloat nematode. Usually, about 5-20 grams of tissues obtained from 5-10 bulbs/sample were processed for the extraction of live stages of the bloat nematode over 3-4 days incubation by the pie-pan method (modified Baermann). Results obtained confirmed the presence of the bloat nematode in 28 samples (31.8% recovery) that were collected from garlic plantings in 16 counties. Infestation levels detected were as high as 987 nematodes/g tissues. These results documented the widespread of the bloat nematode throughout NY and undoubtedly it has been present for several years and spread widely by the propagation, exchange and/or purchase of infected planting materials.

*TYLENCHULUS SEMIPENETRANS* IN CITRUS ORCHARDS ON NEWLY RECLAIMED LAND IN EGYPT. **Abd-Elgawad[1], Mahfouz M.M., L.W. Duncan[2], F.F.H. Koura[1], A.E. Abd El-Wahab[3], S.A. Montasser[3], and M.M.A. Hammam[1].** [1]Phytopathology Department, National Research Center, El-Tahrir St., Dokki 12622, Giza, Egypt; [2]University of Florida, IFAS, Citrus Research and Education Center, 700 Experiment Station Road, Lake Alfred, FL 33850, USA; [3]Department of Agricultural Zoology and Nematology, Faculty of Agriculture, Al-Azhar University, Egypt.

Soil and roots from 20 trees in each of three mature citrus orchards (15-17 years) and one five-year-old orchard in Al-Behera governorate, Egypt were sampled for the citrus nematode, *Tylenchulus semipenetrans,* to determine the relationships between the fruit yield, tree condition and the nematode population levels. There were trends ($r = -0.44$ and $-0.40$; $P=0.052$ and $0.085$) in two of the mature orchards indicating reduced yield with increasing numbers of females infecting the roots. Although the orchards were selected without prior information about nematode infestations, all trees at each site were infected by citrus nematodes and the infection rates exhibited little variability between trees. The standard error to mean ratios for numbers of females per gram of fibrous roots in three orchards were $< 0.10$ and $<0.25$ in the fourth. The average number of females per gram root in the mature orchards ranged from 286 to 445 and was 69 in the young orchard**.** These results and those of previous surveys emphasize the problem of anthropogenic movement of citrus nematodes into newly planted, reclaimed lands via infested planting material and infested soil from old Nile Valley orchards used to improve the quality of desert sands. The adoption of nursery certification protocols and orchard sanitation practices that have successfully excluded citrus pathogens from newly planted regions in Florida could be a highly cost-effective pest management strategy for the reclaimed areas of Egypt.

NEMATICIDAL EFFECT OF MONOTERPENE CONSTITUENTS OF ESSENTIAL OILS TO *CAENORHABDITIS ELEGANS*. **Abdel-Rahman, Fawzia H.[1], N. M. Alaniz[1], B. Wilson[1], E. Mansoor[1,] S. Deolu-Sobogun[2] and M. A. Saleh[2].** [1]Department of Biology and [2]Department of Chemistry, Texas Southern University, Houston, Texas 77004.

Monoterpenes are found in the essential oils (natural volatile compounds) of many plants. The toxicity of thirty three monoterpenes and one sesquiterpenes (farnesol) of essential oils was evaluated using the model nematode *Caenorhabditis elegans* in the bioassays. The Wild type N2 *C. elegans* (originally obtained from Caenorhabditis

Genetic Center (CGC) at University of Minnesota (Minneapolis, MN), was cultured on NGM plates seeded with *Escherichia coli* OP50 as a food source. Nematodes were washed from NGM plates and prepared for the bioassay in M9 buffer solutions. All tested terpenoids were prepared in DMSO to serve as a vehicle; the final concentration of each tested chemical was 50 ppm (50μg/ml) after it was mixed with 2 ml of nematode /buffer mixture, Each 1 ml of the M9 buffer/nematode suspension contained about 1000 different developmental stages of *C. elegans*. Twenty four-well plates were utilized for the bioassay, and each treatment was replicated 4 times, *C. elegans* in DMSO only served as control. All plates were incubated for 24 h at 21 °C. After the incubation period all nematodes in all treatments were transferred to M9 buffer solution only and incubated at 21 °C for another twenty four hours. The dead and living nematodes were counted using the stereomicroscope (*C. elegans* considered dead when it did not respond to repeated touch with a probe). Mortality was determined for each tested chemical and the control. Mortality varied tremendously between different tested terpenoids. α-terpinene, eugenol, pseudoionone and thymol killed 87%, 83%, 87% and 89% respectively of the tested population of *C. elegans*. Linalyl acetate, cinnamaldehyde, α-pinene, verbenone and citronellic acid showed low toxicity and killed only 18%, 24%, 13%, 18% and 6% respectively within the *C. elegans* bioassay; death in control was 15%. The terpenpoids that demonstrated high toxicity toward the tested organism *C. elegans* were tested further to determine their $LC_{50}$. Five different concentrations from 8 ppm to 135 ppm (8μg/ml-135μg/ml) were prepared from each tested chemical, the bioassay was run as described previously and the $LC_{50}$ was determined graphically by plotting concentrations versus mortality. Some chemicals demonstrated very low $LC_{50}$ (highly toxic), such as thymol, geraniol, and α-terpenine with $LC_{50}$ of 13, 14, and 15 ppm respectively.

DIVERSITY AND DISTRIBUTION OF ENTOMOPATHOGENIC NEMATODES (HETERORHABDITIDAE AND STEINERNEMATIDAE) IN EGYPT. **Abu-Shady[12], Noha M., M.M. Shamseldean[1], N.A. Abd-Elbary[1] and S.P. Stock[2].** [1]Dept. of Zoology and Agricultural Nematology, Faculty of Agriculture, Cairo University, **12613 Giza,** Egypt; [2]Dept. of Entomology. University of Arizona, 1140 E. South Campus Dr.,Tucson, AZ 85721,USA.

At present, there is scatter information on the diversity and natural occurrence of entomopathogenic nematodes in Egypt. Therefore, this study reports results from a survey conducted in Egypt which considered this country's four natural geographic regions: a) northern (Mediterranean Sea coast and Nile Delta), b) middle (Nile valley), c) southern and d) Sinai Peninsula regions. These regions have different climate, soil types and crops. Overall a total of 1,000 soil samples were collected. Of them, more than 180 samples (18%) were EPN-positive. Currently, 100 isolates have been molecularly characterized to species level. ITS rDNA sequence analysis was considered as an initial screening/diagnostic approach. Molecular analysis revealed that 58% of the EPN positive samples contained nematodes in the family Heterorhabditidae and the remaining 42% of the samples yielded nematodes in the Steinernematidae. All recovered *Heterorhabditis* isolates were identified as *Heterorhabditis indica* Poinar, (Karunakar and David, 1994). Among steinernematids, isolates were placed mainly into two species: *S. abbasi* Elawad et al., 2002 and *S. carpocapsae* (Weiser, 1954). In addition to these results, one sample from Aswan governorate (southern region) yielded a *Steinernema* species that is currently under study. This nematode is a member of the *glaseri*-group and is closely related to *S. arenarium*. Species in the *glaseri*-group have been reported from Mediterranean countries including Italy, Morocco and Israel. Apparently, this isolate is a novel species, however further studies are warrant to confirm its complete identification. Surprisingly, EPN fauna in Egypt does not seem to be very diverse with respect to number of species. We speculate factors such as climate and soil types limit species diversity. However, those EPN species such as *S. abbasi* and *H. indica* which are adapted to hot and dry climate conditions prevail and are widely distributed.

IN-VIVO GENE EXPRESSION REVEALS DIFFERENCES IN MOLECULAR FEATURES USED BY *PHOTORHABDUS* AND *XENORHABDUS* FOR VIRULENCE AND SYMBIOSIS. **An, Ruisheng and P.S. Grewal.** Department of Entomology, Ohio State University, Wooster, Ohio 44691.

Associations between *Photorhabdus* and *Xenorhabdus* bacteria and their respective nematode hosts *Heterorhabditis* and *Steinernema* share a common biological function, allowing the partnership to endure, infect, kill, and reproduce within an insect host. The bacteria rotate between being a nematode symbiont and an insect pathogen, which presents unique opportunities to study the mechanisms of microbial virulence and symbiosis as a whole. Here, we performed comparative analysis of molecular mechanisms of virulence to an insect and symbiosis with the nematode partner in *Photorhabdus* and *Xenorhabdus*. Using SCOTS technique, we identified 40 genes in *P. temperata* and 39 in *X. koppenhoeferi* which are up-regulated in the hemolymph of the white grub *Rhizotrogus majalis*. Up-regulation of these genes specific in either one of the bacteria was confirmed by comparative hybridization. More than 60% of the identified genes were unique to each bacterial species suggesting vastly different virulence mechanisms used by the two species. For example, except *tcaC* and *hemolysin* related genes, other virulence genes were different between the two bacteria. TCA cycle genes were up-regulated in *P. temperata*

whereas those involved in glyoxylate pathway were up-regulated in *X. koppenhoeferi*, indicating differences in metabolic adaptation between the two bacteria. Up-regulation of genes encoding different types of nutrient uptake systems further emphasized the differences in nutritional requirements of the two bacteria. *P. temperata* displayed up-regulation of siderophore-dependent iron uptake system, but *X. koppenhoeferi* up-regulated siderophore-independent ion uptake system. Further analyses of these identified genes have indicated possible mechanistic associations between the identified gene products in metabolic pathways, providing an interactive model of pathogenesis for each bacterium species. We also used SCOTS technique to uncover mechanisms of symbiotic persistence of the bacteria in the nematode partner. Analyses of in-vivo differentially expressed genes by *P. temperata* and *X. koppenhoeferi* reveal key molecular features reshaped by the bacteria to persist in the enduring infective juveniles. In addition to starvation responses, both bacteria adopt major physiological shifts in the infective juvenile minimizing their nutritional dependence on the nematode. Both bacteria appear to maintain their cytoplasmic $H^+$ level via differential regulation of proton transport systems. While *Photorhabdus* utilizes pentose phosphate pathway in the nematode, glyoxylate pathway is likely preferred by *Xenorhabdus*. Although both bacteria retain flagella production ability, motility appears to be paralyzed in both and biofilm is developed only in *Photorhabdus*. Mutational analysis on five selected *Photorhabdus* genes which represent different cellular processes further reveals that such transcriptional reshaping is critical for bacteria to persist in the nematode infective juvenile. Overall, our investigations demonstrate that the closely related *Photorhabdus* and *Xenorhabdus* have developed vastly different molecular mechanisms for the same goals of virulence to insect and symbiosis with the nematode.

NEMATODE AND FUNGAL COMMUNITIES ASSOCIATED WITH MANGO DECLINE OF SOUTHERN PUNJAB. **Anwar[1], Safdar A., M.V. McKenry[2] and H.A. Ahmad[1].** [1]Institute of Plant pathology, University of the Punjab, Quaid-e-azam Campus, Lahore,-54590, Pakistan; [2]Department of Nematology, University of California, Riverside CA 92521, USA, FMC Private Limited, Rahim-Yar-Khan, Pakistan.

Plant parasitic nematodes and fungal pathogens associated with decline of mango (*Mangifera indica* L Family-Anacardiaceae) were assessed within southern Punjab plantations. Trees in decline exhibited symptoms of arrested root systems with reduced numbers of feeder roots. Such trees had chlorotic and necrotic leaves, leaf abscission from branch tips, bare terminal twigs and dead branches. The appearance or nonappearance of mango decline was recorded for 250 mango trees in 17-mango-orchards. The severity of decline foliage symptoms was sorted on a 1 to 5 scale. The mean disease severity of decline was highest (2.90), intermediate (2.62) and lowest (1.80) on 30%, 50% and 20% of the mango trees, respectively. The fungi most often isolated from symptomatic terminal braches of decline mango trees were included, alphabetically, *Alternaria alternata*, *Botryodiplodia theobromae, Ceratocystis fimbriata Colletotrichum gloeosporioides, Fusarium* spp. and *Nattrassia mangiferae*. The relative abundance of recovery of fungi varied among the mango cultivars and source of sampling. Three fungal pathogens including *B. theobromae, C. fambriata,* and *N. mangiferae* were also identified from roots, barks and vessels collected from the stem and the collar region of mango decline trees. Incidence of *B. theobromae* [(number of samples with *B. theobromae* ÷ total number of samples) × 100] involved 87% of sampled trees. By contrast, incidence of *C. fambriata* and *N. mangiferae* was relatively low (ca 5.5%). Plant parasitic nematodes associated with declining trees included *Criconemella sphaerocephala, Helicotylenchus dihystera, Hemicriconemoides mangiferae, Hoplolaimus indicus, Meloidogyne* spp., *Pratylenchus brachyurus,* Rotylenchulus reniformis, *Trichodorus* spp., *Tylenchorhynchus claytoni, Tylenchus filiformis* and *Xiphinema* spp.. Populations of *H. mangiferae, Macroposthonia* spp., and *R. reniformis* were very high. Possible factors contributing to mango decline must include *H. mangiferae and R. reniformis*. Association of fungi with tissues symptomatic of mango decline in the presence of none to high population levels of plant parasitic nematodes is an area in need of further study to understand the relationship between nematodes and fungal pathogens in inducing decline syndrome in mango.

INDUCED RESISTANCE TO MELOIDOGYNE INCOGNITA AND ROTYLENCHULUS RENIFORMIS IN COTTON. **Aryal[1], Sudarshan K. Richard F. Davis[2], Katherine L. Stevenson[1], Patricia Timper[2], and Pingsheng Ji[1].** [1]Department of Plant pathology, University of Georgia, Tifton, GA; [2]USDA-ARS, Crop Protection & Management Research Unit, Tifton, GA.

Induced resistance against plant-parasitic nematodes has been documented, but is not as well understood as induced resistance to other pathogen groups. Our objective was to determine whether co-infection of cotton by *Meloidogyne incognita* and *Rotylenchulus reniformis* affects the population level of either nematode compared to infection by each species individually. A series of split-root trials was conducted; each trial included four treatments and 10 replications in a randomized complete block design. The four treatments were single plants inoculated with 1) *R. reniformis* only, 2) *M. incognita* only, 3) both *R. reniformis* and *M. incognita*, and 4) a non-treated control. The root system of each plant was divided to allow root growth in two adjacent pots, with each pot containing 750 $cm^3$ of steam-pasteurized soil. One half of the root system of 6-week-old plants was inoculated with *R. reniformis*

on day 0 and other half was inoculated with *M. incognita* on day 0 or day 14 depending on the experiment. Experiments were conducted on DP 0935 B2RF (susceptible to both nematodes) and LONREN-1 (resistant to *R. reniformis*). Experiments were terminated 8 weeks after inoculation with challenge inoculum, and both soil (vermiform extraction) and roots (egg extraction) from each half of the root system were processed to determine the total nematode population levels. Root galling was rated on a 0 to 10 scale. Mixed models analysis and comparison of least squares means indicated no significant differences in root galling (except on LONREN-1) or population levels when the two nematode species were introduced on the same day. However, when *M. incognita* was introduced 14 days after *R. reniformis*, reductions in galling (36 % on DP 0935 and 33 % on LONREN-1) and *M. incognita* population levels (34 % on DP 0935 and 45 % on LONREN-1) were significant ($P \leq 0.05$) on plants that were inoculated with both nematodes compared to plants that were inoculated only with *M. incognita*. The effect of *R. reniformis* and *M. incognita* on induction of enzymes involved in systemic acquired resistance (P-peroxidase, G-peroxidase, and catalase) also was investigated in DP 0935 and LONREN-1. Activities of all three enzymes were higher ($P \leq 0.05$) in leaves of *M. incognita* and *R. reniformis* treated plants than in the leaves of control plants, except that *M. incognita* had no catalase activity on LONREN-1, and the effects were usually apparent 6 days after treatment. This study documents for the first time that infection of cotton by a nematode can elicit enhanced defense to another nematode species through induction of systemic acquired resistance.

DO AMBUSH AND CRUISE FORAGING NEMATODES DISPERSE DIFFERENTLY IN THE ABSENCE OF HOSTS? **Bal, Harit K., R.A.J. Taylor, and P.S. Grewal.** Department of Entomology, OARDC, The Ohio State University, Wooster, OH 44691.

Lack of information on population biology of entomopathogenic nematodes (EPNs) is a major obstacle in establishing sustainable pest management programs and conservation approaches for using these biological control agents. It is critical to understand EPN spatial and temporal dynamics to replete this gap in knowledge. Laboratory studies have identified dichotomy in foraging behavior of EPN species but little is known about the impact of these strategies on their dispersal in the field in the absence of hosts. We compared the rate of lateral movement and population dispersal pattern of the ambusher, *Steinernema carpocapsae* and cruiser, *Heterorhabditis bacteriophora* from infected host cadavers in sterilized silt loam soil (24% moisture) in microcosms (0.05 m2 to 1.5m2) with or without vegetation. Soil core samples (5 by 2 cm dia) were taken at different intervals (6 to 240 h) and distances (7 to 61 cm) from the cadavers and nematode movement was estimated by baiting with *Galleria mellonella* larvae. All experiments were replicated 5 times and repeated. Spatio-temporal data were analyzed using two-dimensional modified Fick Diffusion Model with least squares method, the parameters of which were adjusted for best fit. The two species differed in the pattern of dispersal spatio-temporally, with nearly all *H. bacteriophora* moving some distance away from the cadaver, but most *S. carpocapsae* remaining close to the cadaver, with about 1.3 % moving faster and farther than *H. bacteriophora* at almost all intervals. The average population displacement was similar (5.8-6.0 cm/day) for both species. Vegetation had no impact on *S. carpocapsae*, but it enhanced *H. bacteriophora* dispersal. This study reveals remarkable innate capacity of EPNs to move distances over 100 times greater than their body length in a day in the soil with no hosts. Further investigation on the dispersal pattern of the two species in the presence of host or non-host insects will be conducted mimicking the natural field conditions. Such quantitative understanding of EPN dispersal is critical for designing strategies for establishing sustainable populations of these important biological control agents.

MANURE AND CHEMICAL FERTILIZER EFFECT ON SOYBEAN CYST NEMATODE, NEMATODE COMMUNITY, AND CROP YIELD IN SCN-SUPPRESSIVE AND CONDUCIVE SOILS**.** **Bao, Yong, J. Vetsch, S. Chen, and G. Randall.** University of Minnesota Southern Research and Outreach Center, 120th Street, Waseca, MN 56093.

Soybean cyst nematode (SCN), *Heterodera glycines*, is the major yield-limiting pathogen of soybean. Field experiments were conducted in 2009 and 2010 to determine the effects of liquid swine manure and chemical fertilizer P and K on SCN, nematode communities, and soybean and corn yields in an SCN-suppressive site and a conducive site at Waseca, Minnesota, USA. The experiments were arranged in a split plot design with three crop sequences as main plots and three fertilizer treatments as subplots. The crop sequences included SS, RS, and CS, where S is SCN-susceptible soybean, R is SCN-resistant soybean, C is corn; and the first and second letters represent crops in 2009 and 2010, respectively. The fertilizer treatments were liquid swine manure at 37.4 $m^3$/ha, 49 kg P/ha, 93 kg K/ha, and no fertilizer. The manure was injected into the soil 10 cm under each intended row with 30-cm wide sweep injector two weeks before planting. The PK fertilizer was broadcast on the soil surface and then incorporated into soil before planting in 2009. Soil samples were collected prior to applying fertilizers, 45 days after planting, midseason, and at harvest to determine population densities of SCN eggs and second-stage juveniles (J2), and individual genera in the nematode community. Soybean and corn yields were measured. The

manure treatment reduced the J2 population density in the SCN-suppressive soil at 45 days after planting, whereas fertilizer treatments did not affect SCN egg population density. The application of manure reduced the percentage of plant-parasitic and fungal-feeding nematodes, and increased bacteria-feeding nematodes. The nematode guild of $Ba_1$ responded to manure dramatically and immediately. The higher values in Dominance and EI, and the lower values in Diversity, $\sum$MI, FFB, and CI occurred when manure was applied in both SCN-conducive and suppressive soils. Significant effect of fertilizers on soybean yield was observed in the SCN-conducive soil but not SCN-suppressive soil. Compared to no-fertilizer treatment, the soybean yield increased 638 kg/ha by manure, and 361 kg/ha by PK in 2009; 351 gk/ha by manure, and 294 kg/ha by PK in 2010. The fertilizer treatment effect on soybean yield was greater for SCN-susceptible soybean than resistant soybean in the SCN-conducive soil; yield difference between the SCN susceptible soybean and resistant soybean was greater in no-the fertilizer treatment than PK and manure treatments. The corn was harvested at 13,378 kg/ha in manure-treated plots, 44.5% increase in yield compared to other fertilizer treatments. The concentration of nitrate in the soil treated with manure was over triple of that in the soil treated with PK and no fertilizer. Manure application increased ammonium and zinc, while chemical fertilizer increased phosphorus concentration in soil. This study suggests that soil fertility management, especially application of manure, is a useful strategy to alleviate SCN damage, and increase crop yields.

PEERING INTO THE BLACK BOX: BUILDING AN UNDERSTANDING OF THE POPULATION BIOLOGY OF ENTOMOPATHOGENIC NEMATODES. **Barbercheck, Mary. Pennsylvania State University**

Soils have often been viewed as a black box – life in the soil has been difficult to understand and manipulate because of the complexity and heterogeneity of the processes and interactions occurring there. Entomopathogenic nematodes (EPN) have been studied intensively because of their role as natural mortality factors for soil-dwelling arthropods and their potential as biological control agents for soil-dwelling insect pests. Even though many aspects of the biology and ecology of EPN are well-studied, the reasons that underlie their occurrence and persistence (or failure to persist) in soil are not fully understood. This is in part due to research that has focused on biotic and abiotic effects on nematodes removed from their natural environment. Despite the widespread occurrence of EPN on a global scale, EPN populations at local scales in managed and unmanaged systems consistently vary in both space and time, and are difficult to predict. For effective biological control, population density must be managed to provide sufficient control when and where insect pests occur. In managed systems, habitat and populations can be designed, but the level of effort required and expected benefit in terms of pest suppression can be predicted only if EPN population dynamics can be adequately described and predicted. Soil is a dynamic system and, except for attention to the physical environment, e.g., soil moisture, many attempts to apply and manage EPN for biological control have been made without adequate consideration of the ecology of EPN-environment interactions, and have resulted in inconsistent suppression of target host populations. In addition to physical and chemical factors (e.g., soil texture and structure, soil water status, gases, temperature, pH), numerous biotic factors (e.g., prey population, food-plant of herbivorous prey, intraguild predation, alternate prey) influence predator-prey, host-parasite, and other food web interactions. Human actions influence these interactions through agricultural production practices such as tillage, irrigation, pesticide and fertilizer application, and crop variety selection. The commercial production and use of EPN raises many questions regarding population dynamics. What is the best strategy for augmentation to maximize control of particular insect pests in particular environments? When and where is the conservation of endemic nematodes a viable alternative to augmentation for pest management? In this presentation, I will review some of the key research that has contributed to our understanding of soil and nematode ecology, on persistence, efficacy and spatio-temporal dynamics of EPN, the implication of this information for the application and conservation of EPN, and some areas for future research to further our ability to utilize them effectively for biological control.

DISCOVERY OF VIRUSES IN THE SOYBEAN CYST NEMATODE: FLU OR FLUKE? **Bekal, Sadia, Niblack, T., Domier, L. and Lambert, K.N.** University of Illinois at Urbana-Champaign, 1102 South Goodwin Ave. Urbana IL 61801

Nematodes are the most abundant multi-cellular animals on earth, yet little is known about their viral pathogens. Consequently, nematode viruses have been overlooked as important biotic factors in the study of nematode biology. In a project to sequence the transcriptome of *Heterodera glycines*, the soybean cyst nematode (SCN) four different RNA viruses were identified. The nematode virus genomes were discovered by high-throughput sequencing and assembly of cDNA derived from SCN eggs and second-stage juveniles. The virus genomes were very abundant in the cDNA suggesting they were replicating in the nematodes and were not just physically associated with the nematode. All four viruses had negative-sense RNA genomes, and were distantly related to nya- and bornaviruses, rhabdoviruses, bunyaviruses, and tenuiviruses. Some members of these families replicate in and are

vectored by insects, and can cause significant diseases in animals and plants. Initial experiments indicate that the SCN viruses do not replicate in soybean. The novel viral sequences were detected in all life stage of SCN, but their presence in SCN eggs suggests these viruses are transmitted vertically. While there was no evidence of integration of viral sequences into the nematode genome, we detected mRNA transcripts from these viruses by using QPCR confirming their replication in the nematodes cells. Transmission electron microscopy also confirms the presence of virus particles in highly infected SCN females. This data is the first finding of virus genomes in parasitic nematodes. This discovery highlights the need for further exploration for nematode viruses in all tropic groups of these diverse and abundant animals, to determine how the presence of these viruses affects the fitness of the nematode, strategies of viral transmission and mechanisms of viral pathogenesis. If these nematode viruses have proper characteristics, they may ultimately be useful as biological control agents or as vectors to manipulate nematode gene expression.

INVESTIGATIONS INTO THE RELATEDNESS OF THE NEMATOPHAGOUS FUNGI *DACTYLELLA OVIPARASITICA* AND ARF-L. **Smith Becker, Jennifer[1], J. Yang[2], J. Borneman[2], P. Timper[3], R.R. Riggs[4], and J.O. Becker[1].** Departments of [1]Nematology, [2]Microbiology and Plant Pathology, University of California, Riverside, CA 92521, [3]USDA ARS, P.O. Box 748, Tifton, GA 31793, [4]Department of Plant Pathology, University of Arkansas, Fayetteville, AR 72701.

The fungus *Dactylella oviparasitica* is considered the causal agent of a sugar beet cyst nematode suppression in a field at the Agricultural Operations, University of California, Riverside. The sterile nematophagous fungus ARF-L with similar mycelium appearance and biocontrol activity against the soybean cyst nematode *Heterodera glycines* was the subject of a series of investigations at the University of Arkansas starting in the 1990s. We compared in vitro growth, parasitism of *H. schachtii* and genetic relatedness of ARF-L strain TN14 with *D. oviparasitica* strain 50. Both fungal strains infected immature white *H. schachtii* females when plated together on water agar, but neither fungus was able to parasitize viable eggs in vitro. The growth rate of ARF-L on PDA was approximately double that of *D. oviparasitica* at 28°C. The later fungal strain grew fastest between 23°-28°C and was inhibited at higher temperatures, while the growth rate of ARF-L increased up to 30°C. The growth rate of *D. oviparasitica* was similar on water agar and PDA, while growth of ARF-L was reduced by more than 50% on water agar. In a greenhouse experiment with sugar beet seedlings in sandy loam, *D. oviparasitica* reduced the number of white females of *H. schachtii* by 40% after one generation while ARF-L had no effect. The number of cfu of *D. oviparasitica* increased steadily in soil over a period of 6 weeks while ARF-L grew only slightly. Analysis of the 5.8S rRNA gene from ARF-L indicated that the strain is closely related to *D. oviparasitica* strain 50 (97% sequence identity).

CROP ROTATION FOR THE MANAGEMENT OF THE GOLDEN NEMATODE *GLOBODERA ROSTOCHIENSIS* IN CANADA. **Bélair[1], G., L. Simard[2] and B. Mimee[1]**. Horticulture Research and Development Centre, Agriculture & Agri-Food Canada, Saint-Jean-sur-Richelieu (Quebec) Canada J3B 3E6. [2]Syngenta, Basel, Switzerland.

The discovery of the golden nematode (*Globodera rostochiensis*) pathotype Ro1 in Quebec enforced the importance to develop and implement good management strategies against this quarantine nematode to help Canadian potato growers to face this new challenge. The decline of *G. rostochiensis* densities under the following crops was monitored: corn, pearl millet, mustard (green manure), soybean, sticky nightshade, and resistant potato cv. Andover and Tenace (harbouring H1 gene). From 2007 to 2009, the experimentation was performed in field microplots (1 x 2m) installed in two infested sites where the average *G. rostochiensis* densities on susceptible potato cv. Snowden were 50 and 180 viable eggs / g soil on the St-Amable and St-Hyacinthe sites respectively. In St-Amable (sandy soil) after one year, the lowest densities of viable eggs were recorded in the resistant potato plots. Sticky nightshade and mustard provided a control similar to a non-host crop. In St-Hyacinthe (organic soil) after one year, the lowest densities of viable eggs were again recorded following resistant potato. Sticky nightshade provided a control level similar to resistant potato. After three consecutive years of resistant potato and sticky nightshade in St-Hyacinthe, *G. rostochiensis* densities were below the detection level. After three consecutive years of a non-host crop, nematode densities were below the damage threshold of 5 viable eggs / g soil. The use of H1 resistant potatoes has provided the most significant reduction of viable eggs in the soil on the two experimental sites.

NEMATODE BIODIVERSITY AT OTERO MESA, NEW MEXICO, A PRISTINE DESERT GRASSLAND. **Bernard, E.C.[1], J.M. Trojan[2], and S.H. Thomas[2].** [1]Dept. of Entomology and Plant Pathology, University of Tennessee, 2431 Joe Johnson Drive, 205 Plant Sciences, Knoxville, TN 37996-4560, [2]Dept. of Entomology, Plant Pathology & Weed Science, PO Box 30003, MSC 3BE, Las Cruces, NM 88003-8003.

Otero Mesa, a 4,900-km$^2$ region of the northern Chihuahuan desert located in south-central New Mexico, is one of the few remaining pristine, native short-grass prairies in the U.S. The average elevation is 1546 m with an

average of 25 cm of precipitation annually. Shallow (7-10cm) surface soil horizons for both sites are defined as very fine sandy loam. Subsurface soil horizon textures are sandy clay loam approximately to a 30-cm depth, below which exists gravelly sandy clay loam with indurated calcium carbonate fragments. This shallow hardpan acts to retain any seasonal moisture and inhibit the invasion of deep-rooted shrub plants. Otero Mesa an ecologically unique landscape, containing one of the largest tracts of black grama grassland remaining in the Chihuahuan desert and remains a vital habitat to a rich diversity of plants and animals not found elsewhere or declining in their range. These attributes have led environmental groups to seek protection of this area from oil and natural gas drilling. Soil samples were collected in June 2010 in a section of Otero Mesa to compare this location with less pristine but otherwise similar sites elsewhere in this ecoregion. Auger cores were taken to a depth of 20 cm and composited. Soils were moistened and maintained at room temperature for 48 hours. Nematodes were extracted by means of elutriation and selected nematodes were processed to provide permanent mounts for identification. The nematode fauna in these samples was of relatively low diversity (15 spp.), consisting of Tylenchida (7 spp.), Dorylaimida (5 spp.), and Cephalobina (3 spp.). Aphelenchoidea and other Rhabditida were not observed. Except for an undescribed amphimictic *Tylenchorhynchus* sp., which was numerically dominant, most nematodes were juveniles. Other Tylenchida consisted of single species of *Boleodorus*, *Merlinius*, *Paratylenchus*, *Pratylenchus*, and *Quinisulcius*, as well as an unplaced species resembling Tylenchidae but having some anguinid characteristics. Among the dorylaimids were a very long *Labronema*-like sp. and a putative nygellid with a basket-like carcharolaimid lip region. Cephalobids were represented by *Acrobeles*, *Cephalobus*, and *Eucephalobus* spp. The generic composition of the Otero Mesa nematofauna bears some resemblance to that reported earlier in other Chihuahuan desert studies. Much more sampling at this site, including deep sampling in rhizospheres, is necessary to determine the dimensions of nematode biodiversity at Otero Mesa.

RAPIDLY ASSESSING NEMATODE BIODIVERSITY USING HIGH-THROUGHPUT SEQUENCING: CASE STUDIES FROM MARINE ECOSYSTEMS. **Bik[1], Holly M., Jyotsna Sharma[2], Kenneth M. Halanych[3], and W. Kelley Thomas[1].** [1]Hubbard Center for Genome Studies, University of New Hampshire, Durham, NH 03824, [2]Department of Biology, University of Texas at San Antonio, San Antonio, TX 78249 and [3]Department of Biological Sciences, Auburn University, Auburn, AL 36849.

Marine sediments harbor vast numbers of eukaryotic meiofauna (organisms 42μm-1mm, such as nematodes, fungi, protists, and other metazoa), yet there is a well-recognized gap in the taxonomic understanding of their biodiversity. Meiofauna perform key ecological roles such as nutrient cycling; however, our sparse knowledge of these organisms precludes any informed mitigation and remediation of environmental impacts such as the Deepwater Horizon oil spill. Driven by fundamental advances in DNA sequencing technologies, new high-throughput platforms (454, Illumina) now make possible to conduct *en mass* meiofaunal biodiversity assessment (metagenetics) within an environmental sample using traditional molecular loci (e.g. ribosomal rRNA). Despite the promising outlook of high-throughput approaches, we currently have a poor understanding ribosomal RNA gene evolution and continue to lack the bioinformatic resources needed for effective interpretation of such large datasets. Within the emerging field of eukaryotic metagenetics, current project are focusing on a number of key questions: 1) How structured are meiofaunal communities across benthic marine habitats? 2) How unique are these communities? and 3) What are the effects of anthropogenic disturbance? To address these questions, we have applied both metagenetic and traditional taxonomic methods to assay eukaryotic communities from diverse marine habitats, including oil-impacted sites in the Gulf of Mexico. The comparison of baseline and post-spill sediment communities will provide the first insight into the environmental impacts of the Deepwater Horizon spill on ecologically important (yet historically neglected) meiofaunal taxa.

RELATIONSHIP BETWEEN GENETIC DIVERSITY AND PRIMARY PRODUCTIVITY; A HETERODERA GLYCINES CASE STUDY. **Bird, G.W.,** Department of Entomology, Michigan State University, East Lansing, MI 48824.

The hypothesis, genetic diversity within *Glycines max* results in increased primary productivity and ability to withstand stress caused by *Heterodera glyines*, was evaluated in a six-year field trial (2000-2005) in St. Charles, MI. Six *G. max* systems were compared: 1) continuous *H. glycines* susceptible cultivar, 2) continuous PI 88788-derived cultivars, 3) continuous PI 548402-derived cutivars, 4) continuous PI 437654-derived cultivar, 5) continuous mixture of susceptible, PI 88788, PI 548402 and PI 437654 cultivars, and 6) three-year rotations with PI 88788, PI 437654 and susceptible cultivars. In general, the results supported the hypothesis, with the greatest primary productivity being associated with the genetically diverse mixture of cultivars and the cultivar rotation system, compared to continuous *H. glycines* susceptible or continuous single source of *H. glycines* resistance cultivars. At the end of the experiment, specific HG Types, ranging from HG Type 0 to HG Type 2.5.7, were associated with specific *G. max* systems. In 2010, the hypothesis was re-evaluated in a field trial in Richmond, MI, using *G. max* systems 1–5

in the presence of an HG Type 2 population of *H. glycines*. The greatest primary productivity was associated with the mixture of cultivars, compared to the susceptible or commercially available resistant cultivars. In a 2010, field trial in Decatur, MI., a commercially available cultivar derived from both PI 88788 and PI 437654 had more than 3-fold greater primary productivity, compared to a *H. glycines* susceptible cultivar in the presence of both *H. glycines* and Fusarium *viguliforme* (Mycota).

THE ROLE OF NUTRITION IN THE DETERIORATION OF BIOCONTROL TRAITS IN *PHOTORHABDUS LUMINESCENS*. **Blackburn, Dana[1], D. Shapiro-Ilan[2], and B.J. Adams[1].** [1]Dept. of Biology 401 WIDB, Brigham Young University, Provo, UT 84602, [2]USDA-ARS, Southeastern Fruit and Tree Nut Research Lab., 21 Dunbar Road, Byron, GA 31008

Entomopathogenic nematodes (EPNs) are important biological control agents that kill their insect host with the help of their bacterial symbiont. When EPNs are isolated and reared in the lab beneficial traits needed for efficacy are often lost. Trait deterioration has been observed in the nematode and its bacterial counterpart. It is unknown what causes these changes; however, at least some are due to genetic processes such as genetic drift, inbreeding, and inadvertent selection. Nutrition may also play a role in observed changes in biological control efficacy. This study aimed to determine the affect nutrition has on the bacterial symbiont over a period of twenty subcultures. *Photorhabdus luminescens* sbsp. *akhurstii* was isolated from its symbiotic partner *Heterorhabditis georgiana* and cultured for 48 hours before being subcultured in various media conditions. Cultures were passaged every 48 hours up to 20 passages. Bacteria were cultured in three different nutritional conditions common to lab use; liquid lipid medium (LLM), nutrient broth, and trypic soy broth + 0.5% yeast extract (TSY). After 5, 10, 15, and 20 passages the cultures were tested for virulence, phase switching, and viability based on cell counts. LLM and TSY promoted an increased growth rate compared to nutrient broth cultures. Furthermore, phase switching was occurred less in LLM and TSY media than in nutrient broth. Future research will investigate the effects of switching deteriorated bacteria from unsuitable media to the most beneficial medium. The results of this study may result in an increased understanding of the role the bacterial symbiont plays in biocontrol efficacy, and aid in determining optimal growth conditions for lab and mass-production of these organisms to prevent or slow the rate of trait deterioration.

VERY LOW OCURRENCE OF THE STEM NEMATODE, *DITYLENCHUS DIPSACI*, IN FIELD PEA GRAIN SAMPLES IN WESTERN CANADA. **Briar, Shabeg, O. Molina, and M. Tenuta.** Department of Soil Science, University of Manitoba, Winnipeg, Manitoba, Canada R3T 2N2.

The stem and bulb nematode, *Ditylenchus dipsaci*, is a quarentinable parasite of many crops. This nematode is primarily a pest in temperate regions and has been reported from Western Canada in the past. The primary goal of this research project was to provide an information base for the development of a strategic approach to limit export access issues due to presence of *D. dipsaci* in yellow peas export shipments. A total of 538 pea harvest samples (mainly yellow) were collected from 151 pea growers from Saskatchewan, Alberta and Manitoba in 2009 and 2010. Only 2% of the samples were positive over the two years of the study indicating a very low frequency of the pest nematode occurrence in the pea harvest samples. Nematode positive samples only occurred in 2009. Infestation levels ranged from 4-1500 nematodes $kg^{-1}$ of pea sample. Positives were from different geographical locations across the Provinces. Field cropping history did not seem related to the occurrence of the nematode though the scarcity of positives precludes definitive conclusions. The presence of *D. dipsaci* in most positive samples was related to the presence of dry flower heads/seeds of weeds, especially Canada thistle (*Cirsium arvense*). Nematodes emanated clearly from Canada thistle dry flower heads recovered from the pea samples. Extractions performed on separated weed seeds and peas showed weed seeds to be a source of the nematode in the positive samples but not the pea grain. A reconnaissance survey for weeds in the summer of 2010 in four commercial fields in Manitoba revealed infestation of Canada thistle flower heads (average 139 nematodes $g^{-1}$) with *D. dipsaci*. Canada thistle collected from two nearby road side locations were also infested with the nematode. Morphology characters of adult nematodes were compared to a reference population from garlic in Ontario. The tail length was smaller while the body length to tail length ratio was greater for *D. dipsaci* recovered from pea harvest and weed survey samples than the reference sample. Amplification of the ITS region using 18s and rDNA 1.58s primers yielded a single fragment of ≈550 bp for all positive samples. The banding patterns from the restriction enzymes RsaI and MspI were similar but the ones generated using HinfI varied among the positives indicating ITS sequence variations among populations in Western Canada. Studies are needed to determine the host range and to confirm that weed seeds are the primary source of nematode occurrence in yellow pea grain shipments. Recently recommendations for splitting from the *D. dipsaci* taxon to new species, including a species infecting Canada thistle have been argued. In light of this all and the variability in HinfI restriction pattern, we recommend detailed evaluation of the specie(s) and race(s) of the nematode occurring in Western Canada.

HOST SUITABILITY OF SELECTED PLANT SPECIES, INCLUIDNG SPICE PLANTS TO *MELOIDOGYNE MAYAGUENSIS*. **Brito[1], Janete A., M. Hao[1], M. L. Mendes[2], and D. W. Dickson[2].** [1]Division of Plant Industry, Gainesville, FL 32614; [2]Department of Entomology and Nematology, University of Florida, Gainesville, FL 32611.

*Meloidogyne mayaguensis* has been found infecting many plant species in Florida, including ornamentals, vegetables, fruits and weeds. The objective of this study was to determine the host suitability of 13 selected plant genotypes to *M. mayaguensis* that included basil (genoves, lemon and long leaf), catnip, dill (bouquet and mamot), endive, garlic, marjoram, mint, parsley (common and Italian), and sage. Each plant was inoculated with 5,000 eggs. Tomato 'Rutgers' was used as a control for determining inoculum viability. The experiment was repeated twice. Gall (GI) and egg mass indices (EMI), reproduction factor and eggs per gram of fresh roots were recorded. All genotypes were susceptible to *M. mayaguensis* except garlic, which had a low level of infection (GI= 1.64, EM= 1.91 and 1.67 eggs per gram of fresh root).

RESULTS OF MULTIPLE YEAR TRIALS WITH CORDON™ AS A POST-PLANT TREATMENT TO MANAGE NEMATODES. **Busacca, John[1], Deb Shatley[2], Jim Mueller[3], Jesse Richardson[4] and Harvey Yoshida[5].** Dow AgroSciences, LLC. [1]Indianapolis, IN 46268, [2]Lincoln, CA 95648, [3]Brentwood, CA 94513, [4]Hesperia, CA 92345, [5]Richland, WA 99352.

Plant parasitic nematodes are important pests of established vines. The increase in their populations over time can result in decreases in vigor and yield and can also result in increased incidence of disease. The withdrawal of Nemacur™ from the marketplace has eliminated an important tool to manage nematodes in permanent crops. This presentation summarizes work that was done over multiple years to demonstrate the efficacy of Cordon™, a new formulation of 1,3-Dichloropropene, as a novel post plant tool to manage nematodes on established vines. Low use rates and concentrations of Cordon™, applied via drip irrigation at bloom and shortly after harvest, significantly reduced nematode populations and did not result in crop injury. *Cordon is a registered trademark of Dow AgroSciences LLC; Nemacur is a registered trademark of Bayer CropScience.*

ENTOMOPATHOGENIC NEMATODES AND THE SEASONALITY OF FOOD WEBS IN FLORIDA CITRUS GROVES. **Campos-Herrera[1,2], Raquel, E. Pathak[1], F. E. El-Borai[1,3], R. J. Stuart[1], J. H. Graham[1] and L. W. Duncan[1].** [1]Citrus Research and Education Center, University of Florida, 700 Experiment Station Rd, Lake Alfred FL 33850 (USA). [2]Instituto de Ciencias Agrarias, CSIC, Serrano 115 dpdo Madrid, 28006 (Spain). [3]Plant Protection Department, Faculty of Agriculture, Zagazig University (Egypt).

Greater understanding of the food web dynamics associated with entomopathogenic nematodes (EPNs) and their natural enemies in various environments could provide insights to enhance the effectiveness of EPNs used for the biological control of insect pests. We employed quantitative real time PCR to measure temporal changes in various components of EPN food webs at different soil depths in four citrus orchards with contrasting environmental conditions in Florida. Two sites were on coarse-textured with well-drained soils on the central ridge. Two additional orchard sites were on finer-textured, moderate to poorly-drained soils in the interior flatwoods. At monthly intervals, population levels of five species each of EPNs and nematophagous fungi (NF) and 2 species of *Paenibacillus* (ectoparasites of EPN) were measured using qPCR in individual reactions. We also counted free living nematodes (FLN), and measured fibrous root mass, electrical conductivity and soil moisture. Over an 18-month period, EPNs were detected in 94.3% of the samples (n = 528). Three EPN species were detected (*Steinernema diaprepesi, Heterorhabditis indica* and *H. zealandica*) were detected, with two and sometimes three sympatric species at each site. Numbers of *S. diaprepesi* were related to the species-specific ectoparasite *Paenibacillus* sp. ($r = 0.416$, $P < 0.001$). The depth distribution of *H. indica* varied seasonally, with more individuals detected below 15 cm depth in winter but more detected above 15 cm in summer. In contrast, *S. diaprepesi* was more evenly distributed throughout the soil profile across seasons. The population flux of *S. diaprepesi* was remarkably synchronous at the two central ridge sites that shared environmental characteristics ($r = 0.95$, $n = 24$, $P < 0.0001$), which suggests that climatic factors night play a major rol. The patterns of *Paenibacillus* sp. and endoparasitic NF at both ridge sites were consistent with the possibility that they might regulate numbers of *S. diaprepesi*. At the two interior flatwoods sites (adjacent to one another in the same orchard), *H. indica* was strongly dominant in a low, poorly drained area, whereas *S. diaprepesi* was dominant in a higher, better drained areas, which suggests possible habitat preferences. The trapping NF were almost exclusively recovered in the surface soil profile (upper 15 cm) where the highest FLN numbers and root mass occurred. By contrast, the depth distributions of the obligate endoparasites *Catenaria* and *Myzocitium* spp. were attributed to seasonal patterns. The temporal EPN soil food web assessment using qPCR will provide new insights useful in applied soil ecology.

NEMATODE ASSOCIATES OF A *FUSARIUM* BIOCONTROL ENDOPHYTE OF COLORADO CHEATGRASS. **Carta[1], Lynn K., A.M. Skantar[1], Z.A. Handoo[1], G. Bauchan[2], and M.A. Baynes[3].** [1]United States Department of Agriculture, ARS-BARC-W, Nematology Laboratory, Beltsville, Maryland 20705, [2]United States Department of Agriculture, ARS-BARC-E, Electron and Confocal Microscopy Unit, Beltsville, Maryland 20705, [3]Department of Forest Ecology and Biogeosciences, University of Idaho, Moscow, ID 83844.

Nematodes were isolated from surface-sterilized stems of cheatgrass, *Bromus tectorum* (Poaceae) in Colorado, grown on *Fusarium* (Hypocreaceae) fungus culture, and identified as *Paraphelenchus acontioides* and *Panagrolaimus artyukhovskii*. These nematodes were found during a survey of endophytic fungi as possible biocontrol agents in eight mid-western and western U. S. states and in British Columbia, Canada. Morphometrics and micrographic morphology of these species were generated to supplement the original descriptions. Tabular morphometrics of females of 23 *Paraphelenchus* species were updated from those in the most recent 1984 publication. Variations in the oviduct within *Paraphelenchus* were detailed from specimens on slides and from the literature. Molecular sequences were generated for 18S and 28S ribosomal DNA for *P. acontioides*. For genus *Paraphelenchus* this represents the first identified species so characterized. These sequences were incorporated into phylogenetic trees with related species. *Aphelenchus avenae* isolates formed a well-supported monophyletic sister group to *Paraphelenchus*. *P. artyukhovskii* was morphometrically similar to the species that lack posterior corpus swelling such as *P. detritophagus*, *P. superbus*, *P. subelongatus*, and *P. rigidus*. Diagnostically important SEM head images that could distinguish species in this group were created that showed discrete, unseparated lips similar to *P. detritophagus*. The stoma of *P. artyukhovskii* was longer than that of *P. detritophagus* however, and possessed the two characteristic teeth for this species.

THE EFFECTOR PROTEIN ENCODING GENE *GR33E05* FROM *GLOBODERA ROSTOCHIENSIS* HAS A ROLE IN PLANT PARASITISM. **Chen, Shiyan[1], S. Lu[2], H. Yu[1], and X. Wang[1,3*].** [1]Department of Plant Pathology and Plant-Microbe Biology, Cornell University, Ithaca, NY 14853, [2]USDA-ARS, Northern Crop Science Laboratory, Fargo, ND 58102, and [3]USDA-ARS, Robert W. Holley Center for Agriculture and Health, Ithaca, NY 14853.

Secreted effector proteins encoded by parasitism genes expressed within the esophageal gland cells of plant-parasitic nematodes are essential for successful parasitism of host plants. The *Gr33E05* parasitism gene is expressed exclusively within the single dorsal gland cell of the potato cyst nematode *Globodera rostochiensis* and encodes two predicated effector isoforms, Gr33E05A and Gr33E05B. Gr33E05A differs from Gr33E05B by an internal insertion of 22-amino acid residues as a result of alternative splicing (AS) of the *Gr33E05* pre-mRNA. Transgenic potato lines overexpressing either form of *Gr33E05* showed an increased susceptibility to *G. rostochiensis* infection, whereas silencing the *Gr33E05* transcripts through host-derived RNA interference resulted in less susceptibility to *G. rostochensis* in transgenic potato lines. These results clearly demonstrate that *Gr33E05* is required for nematode parasitism. Yeast two-hybrid screens identified several potato proteins that potentially interact with both isoforms of Gr33E05. Interestingly, a potato protein that interacted with Gr33E05A, but not Gr33E05B in yeast was also identified, suggesting that AS may play a role in diversifying *Gr33E05* function. The identified protein-protein interactions are being characterized to further elucidate the role of *Gr33E05* in promoting plant parasitism by *G. rostochiensis*.

SNEA253 OF *STREPTOMYCES VENEZUELA* CONTROL ROOT-KNOT NEMATODES ON CUCUMBER IN GREENHOUSE TESTS. **Chen, Lijie[1], C.H. Wan[1], X.F. Zhu[1], Y.Y. Wang[1], J.S. Chen[2], Y.X. Duan[1].** [1]Nematology Institute of Northern China, Shenyang Agricultural University, Shenyang, Liaoning,China,110866; [2]Daqing Branch, Heilongjiang Academy of Agricultural Sciences, Daqing, Heilongjiang, China 163316.

Snea253 is a secondary metabolite in the fermentation product of *Streptomyces venezuela*, which was isolated from the soil by Shenyang Agricultural University, and constitutes the Snea253 extract found to kill phytoparasitic nematodes such as soybean cyst nematode and root knot nematode in vitro in our previous research. Concentrations of Snea253 nematicidal extract at 10.2μl · ml$^{-1}$ killed 50% of target *Meloidogyne incognita* J2 in vitro, and killed 90% J2 at 50μl · ml$^{-1}$, providing a dose-response virulence regression equation of Y = 3.40152X +1.75706, LD50 = 10.15534μl · ml$^{-1}$. To evaluate Snea253 effects on root-knot nematodes in soil, we designed a series of dilutions (diluted 50×, 100×, 200× and in vitro LD50 concentration 10.2μl · ml$^{-1}$) of the Snea253 extract. Water and 1.8% avermectin commercial pesticide were included as negative and positive controls treatments, respectively. Five replicates of each treatment, including controls, were added to the soil in pots of cucumbers grown in the greenhouse and plants were inoculated with *M. incognita*. Then the number of root galls, egg masses of *M. incognita* and disease index (DI=Σ[(Si*Ni)/( N*imax)]*100) were investigated in this paper. The results showed that the 50× dilution of Snea253 had a similar level of root-knot nematode control as avermectins in pot experiments, where compared to water controls the inhibition rate of root gall numbers and egg masses was 90.87% and 91.58% respectively, and avermectins was 92.52% and 91.32%, with no significant difference between them. The other dilutions of Snea253 reduced root-knot nematode infection more than

50% compared with the water control in pot experiments. Further research with the same dilution series showed that the 50× Snea253 treatment resulted in a disease index of 29% compared to the control index of 70.1% in greenhouse experiments. For disease index, 50× Snea253 was almost as effective as avermectins (disease index was 18%, the control effect was 81.44%) in greenhouse experiments, with no statistical significance between them. The 100× and 200× dilutions and the in vitro LD50 treatment showed the also provided disease indices of less than 50% compared to the water control. In summary, we showed that Snea253 can reduce root-knot nematode infection of cucumber roots in greenhouse assays and is worthy of further investigation for its potential in nematode management.

INTEGRATING OF A *STREPTOMYCES* BIOAGENT WITH PESTICIDES TO CONTROL *MELOIDOGYNE INCOGNITA*. **Chen, Ying-Yu, P. Chen, T.T.Tsay.** Dept. of Plant Pathology, National Chung-Hsing University, 250 Kuo-Kuang Rd. Taichug 402, Taiwan.

The chitinolytic actinomycete *Streptomyces saraceticus* (SS 31) (LTM® and Agrosustain™) has been widely used to control plant-parasitic nematodes in Taiwan. Seventy two pesticides were screened *in vitro* in a previous study and the results indicated that 55 pesticides did not inhibit the growth of SS 31. Some pesticides lost their inhibitory effect when SS 31 established its population. Eight pesticides that have different inhibitory levels against SS 31 *in vitro* were chosen for the greenhouse experiments. Pesticides and SS 31 were applied in different orders to control the root-knot nematode *Meloidogyne incognita* on water spinach. In treatment 1 (T1), SS 31 and pesticide were applied at the same time along with nematode. In treatment 2 (T2), the pesticide was applied 2 days after the co-inoculation of SS31and nematode. When SS 31 was applied with copper hydroxide, thiophanate methyl-streptomycin, chlorothalonil, ethoprop or carbofuran , the galling index (GI) on the roots or the number of $J_2$ in the soil was significantly reduced. Ethoprop and carbofuran were compatible with SS 31in *in vitro* assay. When SS 31 was applied with ethoprop, the GI was 0.28 in T 1 and 0.08 in T 2; when applied with carbofuran, the GI was 0.58 in T1 and 0.44 in T2. The rusults showed that SS31 and these 2 pesticides works synergically reducing the root-knot disease, and the synergetical effect was more pronounced in T2. Our study suggested that *Streptomyces saraceticus* (SS 31) could be compatible with the use of pesticides in the field conditions.

ASSESSING SOIL NEMATODE FOOD WEBS AND SOIL PHYSICAL AND CHEMICAL PROPERTIES IN URBAN VACANT LOTS IN A POST-INDUSTRIAL AMERICAN CITY. **Cheng, Zhiqiang, A. Knight, R. Glover, K. Quaye, V. Roman, P. Yadav, K. Sharma, S.S. Grewal, R. Islam, and P.S. Grewal.** Department of Entomology, The Ohio State University, 1680 Madison Ave., Wooster, OH 44691.

Post-industrial cities such as Cleveland (Ohio, USA) have accumulated substantial number of vacant lots due to home foreclosures and urban sprawl over the past two decades. Interest in this type of land has escalated recently due to increased demand for food security in disadvantaged urban neighborhoods. One method of reusing urban vacant lots that has been gaining support is to transform them into community gardens for growing fresh produce. However, whether vacant lots can be safely and effectively converted into community gardens to produce food requires further research, especially with respect to soil quality. The main objectives of this study were to: 1) assess the extent of variation in soil biological, physical, and chemical properties in urban vacant lots; 2) determine the relationships among soil properties to identify effective indicators of urban soil quality; and 3) compare soil quality in urban vacant lots to several other urban and non-urban land use types including turfgrass lawns, community gardens, and field croplands.We measured soil nematode food webs and soil physical and chemical properties in 28 vacant lots in the Hough neighborhood, a relatively disadvantaged community, in Cleveland with the ultimate goal to assess their potential suitability for food production. These vacant lots generally received little shading and appeared rarely mowed, and typically had scattered plant cover (natural vegetation, turfgrass, weedy species) and one or few trees. Each vacant lot was divided into three approximately equal sections (1 center section where the house foundation was roughly at, and 2 side sections) and 9 soil cores were collected from each section. The results revealed huge spatial variability in soil properties within vacant lots. However, there was no significant difference for soil nematode community indices, soil chemical and physical properties between center section and side sections. Thirty-four nematode genera were identified, and nematode abundance ranged from 34 to 988 per 10g soil sample. Significant correlations were found between soil nematode community and soil physical and chemical properties. Notably, soil nematode abundance significantly correlated with $NH_4$-N, microbial biomass, soil organic matter, and active carbon. In addition, nematode combined maturity index significantly correlated with soil organic matter, and active carbon. Principle Component Analysis revealed that vacant lots had less structured soil nematode food webs than turfgrass lawns, but they were not different from community gardens. There were no differences in nematode abundance, genus diversity, and enrichment index among urban vacant lots, turfgrass lawns, and community gardens. In contrast to the conventional belief, the overall similarity in the soil nematode

food web conditions among urban vacant lots, community gardens, and field croplands suggest that the food production potential in urban vacant lots could be at par with agroecosystems.

IDENTIFYING GENES THAT PREVENT MITOCHONDRIAL DNA DELETIONS: A TARGETED GENETIC APPROACH USING *CAENORHABDITIS ELEGANS*. **Clark, Katie A. and D.R. Denver.** Department of Zoology and Center for Genome Research and Biocomputing, Oregon State University, Corvallis, OR 97331.

In humans, large deletions in the mitochondrial genome (mtDNA) are associated with both aging and diseases such as cardiac dysfunction. Similarly to humans, *Caenorhabditis elegans* nematodes also experience age-related accumulation of mtDNA deletion mutations. Despite the prominent role of large mtDNA deletions in human aging and disease, the exact molecular mechanism of mtDNA deletion formation remains unknown. We have designed an efficient approach that combines *C. elegans* RNAi methods with a mutation-accumulation approach (MA) to screen for genes that are involved in the formation or accumulation of large mtDNA deletions. We have created a prioritized list of candidate genes with a potential role in mtDNA replication, mtDNA maintenance, or an association with human mitochondrial disorders. For each candidate gene, we are using an MA line approach and bottleneck to single-worm descent in the presence of RNAi against the candidate gene for 10 generations. Bottlenecking to individual worms at each generation minimizing the effects of selection and allows the nearly neutral accumulation of mutations. Using a long-PCR strategy, we can efficiently screen for the presence of large mtDNA deletions in the entire mtDNA genome from our bottlenecked populations. Our pilot study identified a new mtDNA deletion in nematodes exposed to *mtss-1* RNAi. *mtss-1* encodes a predicted mtDNA single stranded DNA binding protein. This approach provides a novel and effective method for discovering new genes that contribute to mtDNA stability.

THE REGULATION OF SYMBIOSIS IN *PHOTORHABDUS*. **Clarke, David J.** Department of Microbiology, University College Cork, Cork, Ireland.

*Photorhabdus* are a genus of Gram-negative bioluminescent bacteria that belong to the family *Enterobacteriaceae*. *Photorhabdus* are highly virulent against a wide variety of insect larvae whilst also maintaining a mutualistic association with nematodes from the family *Heterorhabditidiae*. The bacteria colonize the gut of a specialized non-feeding stage of the nematode, the infective juvenile (IJ). The IJ is found in the soil where it actively seeks out and infects susceptible insect hosts. Once in the insect the nematode regurgitates their symbiotic bacteria into the insect hemolymph where the bacteria replicate, killing the insect within 48-72h. During this time the IJ develops into an adult hermaphrodite that begins to feed on the bacterial biomass present in the insect. The nematodes reproduce for several generations before environmental signals trigger the formation of a new generation of IJs that must be colonized by *Photorhabdus* before the nematodes leave the insect cadaver in search of new targets. The ability of *Photorhabdus* to convert the insect host into an environment that is capable of supporting nematode growth and development is a key feature of the symbiosis between *Photorhabdus* and *Heterorhabditis*. We have shown that nematode growth and development is associated with the elaboration of a complex secondary metabolism during the post-exponential phase of growth of *Photorhabdus*. Products of this secondary metabolism include, but are not limited to, hydrolytic enzymes (e.g. proteases and lipases), small bioactive molecules (e.g. antibiotics and pigments) and bioluminescence and the role of these products in mutualism has, in some cases, been described. We have also shown that a metabolic switch that requires a functional TCA cycle controls secondary metabolism, and therefore symbiosis, in *Photorhabdus*. Finally, although the molecular mechanisms of this metabolic switch are not fully understood, there is evidence to suggest the involvement of a regulatory network that has both transcriptional and post-transcriptional regulatory inputs.

PHYLOGENETIC RELATIONSHIP AMONG NEMATODES OF THE FAMILY CRICONEMATIDAE USING ITS-1rDNA REGION. **Cordero, M, [1] Robbins, R. [1] and Szalanski, A. [2]** 2011. [1]Department of Plant Pathology. 2601 N. Young Ave. Cralley - Warren Research Lab. University of Arkansas, Fayetteville, AR. 72704. [2]Department of Entomology, AGRI 330B, University of Arkansas, Fayetteville, AR. 72701.

A phylogenetic study on nematodes belonging to the suborder Criconematina is presented. The suborder is composed by three Superfamilies: Criconematoidea, Hemicycliophoroidea, and Tylenchuloidea; genera on each family share common anatomical features that allow taxonomist to identify and to classify them. Genera from Arkansas, California, Missouri, North Carolina and Florida were extracted from soil samples using standard methods or received in NaCl 1M or 95% Ethanol. Some of the species collected belong to the genera *Mesocriconema, Criconemoides, Criconema, Ogma, Xenocriconemella ,Hemiciclyophora, Hemicriconemoides,* and *Caloosia* DNA from individual nematodes from each population was extracted and subjected to PCR using the nuclear rDNA gene primers which amplify ITS-1, rDNA2 and rDNA 1.58s. Amplicons were sequenced in both directions and a molecular phylogenetic analysis was conducted using maximum parsimony and maximum likelihood. In addition, genetic variation within species was also examined.

A *PASTEURIA* BASED BIONEMATICIDE FOR MANAGEMENT OF *BELONOLAIMUS LONGICAUDATUS* ON TURF. **Crow, William T. and J.E. Luc.** Entomology and Nematology Dept., University of Florida, PO Box 110620, Gainesville, FL 32611.

The recent development of in vitro methods for culturing *Pasteuria* spp. has made possible the use of *Pasteuria* as a commercial biopesticide. *Belonolaimus longicaudatus* is the most damaging plant-parasitic nematode to golf course turf in the coastal southeastern U.S. An in vitro cultured *Pasteuria* sp. isolate was shown in previous studies to suppress *B. longicaudatus* in pots. This *Pasteuria* isolate is now the active ingredient in EcoNem™, which was launched commercially in 2010. In that year, six field trials evaluated the effectiveness of different rates and application frequencies of EcoNem for management of *B. longicaudatus* on bermudagrass (*Cynodon* spp.). Parameters included nematode population densities in soil and turf health measurements (root lengths and percent green cover). In all these trials, none of the EcoNem treatments were successful in reducing the numbers of *B. longicaudatus* in soil or in improving turf health. Based on these studies, it is not possible to demonstrate that the current formulation of EcoNem is an effective bionematicide for management of *B. longicaudatus* on turf. However, research on the development of new formulations and application methods continues.

NEMATODE BIODIVERSITY IN SOUTH AFRICAN GOLF COURSES. **Mieke S Daneel[1] and H Fourie[2].** [1]ARC-Institute for Tropical and Subtropical Crops, Private Bag X11208, Nelspruit 1200, South Africa mieke@arc.agric.za, [2]North West University, School of Environmental Sciences and Development, PUK Plant Protection, Private Bag X6001, Potchefstroom 2520, South Africa.

Only a limited number of nematodes surveys have been conducted on turf on golf courses in South Africa. In addition, those surveys only focused on estimating the population levels of plant parasitic nematodes that infect roots of such grasses. No information is thus recorded on the presence and abundance of non-parasitic nematodes that form part of the total nematode complex with the soil-root interface of turf grasses. Therefore, during the present study, both plant parasitic and non-parasitic nematodes that include free living, predator and omnivore individuals were studied, when greens of 11 golf courses situated across South Africa were sampled. In terms of plant parasitic nematodes, nine genera including *Hemicycliophora, Helicotylenchus, Rotylenchus, Hemicriconemoides, Paratrichodorus, Xiphinema, Paratylenchus, Pratylenchus* and *Meloidogyne* were identified while a wide variation of functional guilds including fungivore, bacterivore, omnivore and carnivore guilds were recorded from soil samples. Enrichment and structure indices indicated that most of the nematode-based soil food webs of local golf courses were situated in the A and B quadrants. . The nematode community structures differed for the 11 golf courses although non-parasitic nematodes generally dominated, particularly enrichment families belonging to $Ba_1$. Nematode complex seemed to be area related. Plant parasitic nematodes were related to the poorest turf patches and especially *Hemicycliophora*, *Meloidogyne* and *Pratylenchus* were the most important indicators of turf damage followed by *Xiphinema,* Criconematidae and Hoplolaimidae. *Paratrichodorus* seemed less important in terms of turf damage during this survey.

HIGH-THOUGHPUT 18S RIBOSOMAL AMPLICON SEQUENCING OF A TALLGRASS PRAIRIE NEMATODE COMMUNITY. **Darby, B.J[1]., T.C. Todd[2], and M.A. Herman[1].** Ecological Genomics Institute, [1]Division of Biology, [2]Department of Plant Pathology, Kansas State University, Manhattan, KS 66506.

Nematodes are diverse and functionally important components of the grassland ecosystem, but it is difficult to determine how individual species are influenced by environmental changes, such as altered land use patterns, anthropogenic inputs, or climate conditions. One of the significant methodological challenges is the need for a sensitive and specific method to assess the abundance and distribution of nematode species in their natural environment. For example, burning and chronic nitrogen enrichment of grassland soils alters the relative proportions of several nematode families. However, it is not known whether all species within a family or genus respond to these changes in kind, or if changes in the composition of families are obscurred by several species that all behave differently. We have sampled nematodes from a long-term belowground experiment at Konza Prairie LTER Station with treatments of altered inorganic nitrogen (ambient vs. annual amendment of 10 g N / $m^2$) and burning regime (annually burned vs. non-burned). Nematodes were counted and identified to family prior to pooling and extraction of bulk community genomic DNA (along with co-occurring plant, fungal, and other metazoan tissue). We then amplified a phylogenetically informative region from the 18S rRNA SSU gene and sequenced these amplicons with 454-pyrosequencing (GS FLX Titanium). The purpose of amplicon sequencing is high-throughput identification of nematodes with a taxonomic precision that is not generally possible with traditional specimen-based community assays. We found that amplicon sequencing data generally corresponds to that obtained by traditional specimen-based methods, but has significant challenges that have yet to be resolved. As has been reported by others, artifacts from PCR and sequencing errors are a constant challenge but can be managed with careful bioinformatics. The main discrepancy we found between specimen-based and sequence-based

counts is the overestimation of enrichment-type bacterivores, primarily in the family Rhabditidae. We empirically determined that this discrepancy could be attributed primarily to variation in genomic 18S operon copy numbers, and to a lesser extent cell number per individual. Most Rhabditidae species tested thus far appear to have 5 to 10 times more 18S ribosomal operons per individual than non-Rhabditidae species. We conclude that this approach has potential for high-throughput identification of nematodes to near species-level resolution, but it will be challenging to make taxon abundance estimates quantitative. Additional work is needed to improve the quantitative capabilities of high-throughput community amplicon sequencing, including 1) develop methods to reduce PCR and sequencing errors, 2) curate accurate databases of sequences linked to correctly identified nematode species, and 3) estimate ribosomal operon copy numbers for a variety of nematode groups.

GENOMIC RESPONSE OF RHABDITIDAE NEMATODES TO THEIR BACTERIAL PREY. **Darby, B.J., D. Wheeler, M.A. Herman.** Ecological Genomics Institute, Division of Biology, Kansas State University, Manhattan, KS 66506.

Bacterial-feeding nematodes of the family Rhabditidae inhabit soils that are enriched with organic nutrients and tend to have greater concentrations, but typically reduced richness, of bacteria than non-enriched soils. It has been known for some time that the total abundance of enrichment-type bacterivores is largely the result of increased concentration of bacteria, but it is unknown whether the species composition of Rhabditidae in the field is influenced by the species composition of the consumed or associated bacteria. Because bacterial communities in the soil are more complex than can be reproduced in the lab, we propose a genomic approach for understanding the complex relationships between nematodes and the bacteria they consume as prey. In essence, genomic tools allow us to 'ask the nematodes' how they perceive different bacteria and what cellular functions are utilized when exposed to certain bacterial types so that we can extrapolate modeled conditions in the lab to specific soil environments in the field. Although bacteria are a dietary necessity, there is increasing evidence that some bacteria can be pathogenic and should be viewed as potentially adverse components of a nematode's biotic environment. Specifically, we ask 1) how do nematodes respond at a genomic level to different bacteria as prey, 2) is the pattern of gene expression idiosyncratic for each bacterial species, or are there general patterns that correspond to fitness or bacterial traits, and 3) do individual nematode species differ from each other in their response to bacteria? We have sequenced partial transcriptomes of four representative Rhabditidae nematodes from the native Konza Tallgrass Prairie (*Oscheius tipulae, Oscheius sp.* FVV-2, *Rhabditis sp.*, *Mesorhabditis sp.*) into a hybrid assembly of long-read (454 GS FLX Titanium) and short-read (Illumina GAIIx) RNA-seq libraries. As predicted, the proportion of genes with confident homology to *C. elegans* depends on phylogenetic proximity. In addition, there are numerous gene transcripts from native isolates that appear to be lineage-specific without clear homologs in any other available nematode genome sequences. The nematodes that were used for short-read RNA-seq were exposed to six different bacteria (three gram-negative γ-Proteobacteria and three gram-positive *Bacillus* species) in duplicate, and the twelve different samples were uniquely barcoded which allowed digital gene expression profiles for each bacterial treatment to be generated. Preliminary analysis suggests that bacterial-responsive genes are expressed in a number of patterns. While some genes were expressed specifically in response to one or a few bacteria, many had expression patterns that appeared to reflect some bacterial quality such as pathogenicity or gram staining. Specifically, some genes were preferentially expressed in response to benign bacteria, whereas otherws upregulated in response to "adverse" bacteria; still others appeared to be predominantly expressed in response to gram-negative γ-Proteobacteria while others were predominantly expressed in response to gram-positive *Bacillus* species. As these tools continue to be developed for a broader range of ecologically important species, we hope to apply this technology in the field to understand what stresses nematodes must respond to, and how this affects their abundance and distribution.

DO MUCIN-LIKE MOLECULES HAVE A ROLE IN THE ATTACHMENT OF *PASTEURIA PENETRANS* TO THE CUTICLE OF PLANT-PARASITIC NEMATODES? **Davies[1], Keith G., J.A. Khan[1,2], U. Rao[1,2].** [1]Plant Pathology and Microbiology, Rothamsted Research, Harpenden, Hertfordshire, AL5 2JQ, UK, [2]Indian Agricultural Research Institute, Pusa Campus, New Delhi, 110012, India.

Mucin-like molecules belong to a family of glycosylated proteins that are high in serine and threonine in which up to 85 percent of their molecular weight is made up of carbohydrate. They are produced by epithelial tissues and can be in secretions or membrane bound, functioning as lubricants and forming barriers. They also are involved in cell signalling and in immunity. In animal-parasitic nematodes they are present in the cuticle surface coat and play an important role in evasion of the host animals' immune system but very little is known about them in plant-parasitic nematodes, although mucins identified in *Toxocara canis* have putative orthologs in plant-parasitic nematodes. Mucin-like proteins have also been identified in *Caenorhabditis elegans* and RNAi knockdown of these genes has been associated with changes of lectin staining of the nematode cuticle surface. Orthologs to mucin-like genes from *C. elegans* were identified in *M. incognita* amplified by PCR, cloned, sequenced and dsRNA synthesised

by *in vitro* transcription. RNAi knockdown was performed by oral feeding, and *Pasteuria* endospore attachment assays performed at 4, 12 and 24 hours post feeding to test the hypothesis that mucin-like genes of the cuticle surface of infective juveniles have a role in endospore attachment. Endospore attachment tests revealed the orthologus mucin-like gene H43E16.1 increased endospore attachment, whereas C26G2.2 decreased endospore attachment and the effects were time dependent. Orthologus genes to K11D12 and F35E12 had no effect. The results will be discussed from the hypothetical view that a velcro-like mechanism is involved in endospore attachment and that mucin-like molecules play a role in endospore attachment and specificity.

REDUCTION OF ENDOSPORE BINDING OF *PASTEURIA PENETRANS* TO THE CUTICLE OF *MELOIDOGYNE INCOGNITA* BY PEPTIDES PUTATIVELY INVOLVED IN THE DAF-2 SIGNALING PATHWAY. **Davies[1], Keith G., J. Scott[1], J. Rowe[1] and J.E. Hart[2].** Plant Pathology and Microbiology, Rothamsted Research, Harpenden, Herts. AL5 2JQ, United Kingdom, Endocrine Pharmaceuticals, Wilderness End, Tadley, Hampshire, RG26 3TA, United Kingdom.

Micrographic studies of endospores of *Pasteuria penetrans* attached to the surface of second-stage juvniles, show the intimate relationship between fibrous structures on the endospore surface and the nematode cuticle. Peptides derived from a mammalian hormone research project (Haylor *et* al., 2009, *Regulatory Peptides,* 152, 48-53) were shown to increase and decrease fecundity in *Caenorhabditis elegans* at micro-molar concentrations when applied as 14-mer peptides (Davies and Hart, 2008, *Nematology* 10, 103-112). The DAF-2 pathway in *C. elegans* has been shown, amongst others, to have effects on fertility and is part of the innate immune system. Because one of the peptides that is known to inhibit the proliferation of IGF-1 stimulated breast carcinoma cells, and the other is an anagrammatical form of this peptide, we think that the DAF-2 pathway may be involved in fecundity manipulation in *C. elegans*, as it is the equivalent of the IGF-1 pathway in mammals. We therefore hypothesised that these peptides may interact with the nematodes immune system and affect the adhesion of endospores to the cuticle as part of an anti-microbial response. Juveniles were exposed to 14-mer peptides and shortened 6-mers to identify the active centres of the peptides and attachment tests performed. The 14-mers both reduced attachment at 18 and 21 h but by 27 h the attachment was the same as for the controls. In the 6-mer tests EPL016 promoted attachment, EPL036 and EPL037 reduced attachment after 18 h but by 22 h there was little effect when compared to controls. However, EPL037 maintained a low attachment at both 18 and 22 h.

STUDY OF THE VIRUS VECTOR GENUS *TRICHODORUS* (DIPHTHEROPHORINA, TRICHODORIDAE) FROM THE IBERIAN PENINSULA, AN APPARENT CENTER OF SPECIATION. **Decraemer, Wilfrida[1], J.E. Palomares-Rius[2], C. Cantalapiedra-Navarrete[2], B B. Landa[2], I. Duarte[3], M.T.M. Almeida[4], N. Vovlas,[5] and P. Castillo[2].** [1]Royal Belgian Institute of Natural Sciences, B1000 Brussels, Ghent University, B9000 Ghent, Belgium, [2]Institute for Sustainable Agriculture, CSIC, 14080 Cordoba, Spain,[3]Escola Superior Agrária de Coimbra, 3040-316 Coimbra, Portugal,[4]Centre of Molecular and Environmental Biology'Universidade do Minho, 4710-057 Braga, Portugal,[5]Istituto per la Protezione delle Piante, Sede di Bari, CNR, 70126 Bari, Italy.

Trichodoridae are polyphagous root ectoparasites occurring worldwide. Their major pest status is as virus vector of Tobraviruses. Currently, the family has 102 species classified within 6 genera. The genus *Trichodorus* is the largest in number of species (56) and predominantly occurs in temperate regions. Traditional morphology-based taxonomy revealed very high species diversity within the Iberian Peninsula, comprising about one fifth of all Trichodoridae described. Characteristic for this fauna is the presence of a morpho-species group within *Trichodorus*, characterized in males by slightly ventrally curved spicules with a mid-blade constriction with bristles and females with relatively large vaginal sclerotized pieces, quadrangular to triangular in shape. Recent surveys for Trichodoridae in cultivated and natural environments in Southern Spain and compared with the fauna from Portugal revealed four new species of *Trichodorus*, three of them belonging to this morpho-species group. Molecular analyses based on nuclear ribosomal RNA genes (D2-D3 expansion segments of 28S and partial 18S gene) supported not only the new species but also the morpho-species group as a separate clade. The integrated approach of morphology based taxonomy with molecular and biogeographic data enhanced the accuracy of the observed biodiversity and strengthen the hypothesis of the Iberian Peninsula as a center of speciation.

DISPERSAL AND GENE FLOW IN FREE-LIVING MARINE NEMATODES: FROM ECOLOGICAL TO EVOLUTIONARY TIME SCALES. **Derycke, Sofie[1,2], T. Moens[1].** [1]Department of Biology, Marine Biology section, Ghent University, Krijgslaan 281, S8, 9000 Ghent, Belgium. [2]CeMoFE, Ghent University, K.L. Ledeganckstraat 35, 9000 Ghent, Belgium.

Knowledge on the connectivity of populations is important to understand the dynamics and persistence of populations against environmental changes, the relative importance of micro-evolutionary processes for population differentiation and biogeographic distributions of organisms. These processes act along an evolutionary continuum, and can be studied through a population genetics and phylogeographic framework. We here present

the results of such a framework for free-living marine nematodes. Field experiments have illustrated that nematodes frequently occur in the water column suggesting that passive dispersal can be substantial. Successful colonization of habitats then depends on whether dispersal is followed by reproduction (= gene flow), which in turn is influenced by habitat suitability and by the presence of organisms that may hamper the establishment of newly arriving individuals (= priority effects). *Rhabditis marina* typically frequents macroalgae in the intertidal and may therefore be prone to passive dispersal (e.g. through "rafting"). Its high reproductive capacity and short generation time render this species a strong colonizer capable of establishing populations from one or a few gravid females. To understand the colonization dynamics of *R. marina*, we performed a field experiment in which the genetic diversity of newly colonized algae was followed during one month at two contrasting sites in an intertidal saltmarsh. The algal deposits incubated near a source population were more rapidly colonized, reached fivefold higher densities of nematodes and had a higher genetic diversity than those in a site ca 1 km away from the source population. In this remote location, rare haplotypes were abundant suggesting that founder effects and genetic bottlenecks structured these populations. The genetic differences between patches in each site further indicate low effective dispersal because of priority effects. At deeper timescales, populations of nematode species associated with macroalgae showed only slight structuring within an estuary, and restricted gene flow at larger geographical scales. Interestingly, among nematodes associated with macroalgae, opportunistic species (*Rhabditis marina* and *Halomonhystera disjuncta*) as well as species with a long generation time and relatively few offspring (*Thoracostoma trachygaster*) show similar patterns of genetic structure, suggesting that passive dispersal determines their genetic structure. We are currently studying population genetic structure in several endobenthic species and expect a stronger genetic structuring because they are less prone to passive transport than species associated with macroalgae. Finally, our phylogeographic studies have demonstrated that well known biogeographic barriers (Point Conception and the Los Angeles Region) accounted for an additional degree of genetic structuring in *T. trachygaster*, and that Pleistocene glaciations have left a genetic fingerprint in European *R. marina* populations. Shared haplotypes between Europe and North America illustrate that occasional successful long-distance dispersal occurs in marine nematodes. In conclusion, our genetic data show that gene flow in marine nematodes is substantial at scales of hundred kilometers, but restricted at larger geographical scales.

MULTIPLE FAMILES OF PLANT PEPTIDE HORMONE MIMICS ARE ENCODED WITHIN THE COMPLETED RKN GENOMES. **DiGennaro, Peter[1,2], E.H. Scholl[2], and D.McK. Bird[2,3].** [1]Functional Genomics Graduate Program, [2]Plant Nematode Genomes Group, [3]Bioinformatics Research Center, Campus Box 7253, NC State University, Raleigh, NC 27695.

Using a hardware accelerated double-affine Smith-Waterman algorithm, we mined the completed root-knot nematode (RKN: *Meloidogyne* spp.) genomes for sequences with similarity to plant peptide hormones. In contrast to biochemical approaches, this computational screen makes no *a priori* assumptions about transcript abundance or location within the nematode. The recent discovery that the mode of action of ivermectin is to suppress secretion from the SE gland (Moreno *et al.* PNAS, 2010) underscores the importance of secretions beyond those of pharyngeal origin. In *M. hapla* and *M. incognita* we computationally identified 31 genes encoding peptide hormone mimics including members of the CLE and CEP families. *In planta* these regulators have emerged as key determinants of diverse aspects of cellular proliferation and specification. We hypothesize that the RKN-encoded mimics recapitulate these functions as part of the parasitic interaction, including giant cell and gall formation, and suppression of host defense responses. Consistent with the products of these genes being secreted directly into the host apoplast as active molecules, they contain a secretion signal sequence with a predicted cleavage site directly amino to the active domain. This is in contrast to the native CLE and CEP genes which encode a regulatory 'pro' domain between the signal sequence and the active peptide (mass spectrometry shows this is proteolytically cleaved in the apoplast). By eliminating this 'pro' domain, evolution has presumably equipped the nematode to overcome the endogenous regulation of these potent ligands. Based on biological activity plant CLEs have been classified as either 'A' (such as CLV3), which inhibit cell proliferation by antagonizing the WUSCHEL transcription factor, or 'B' CLEs which inhibit *Zinnia elegans* tracheary element differentiation. A and B CLEs are agonistic, with class 'A' potentiating the activity of class 'B' peptides. Based on sequence similarity, in particular conservation of specific residues known through alanine scanning mutagenesis to be critical for function of native CLE peptides, both classes are represented in RKN, suggesting that these animals encode a comprehensive array of plant regulatory functions. Using qRT-PCR we have confirmed the presence of transcripts corresponding to the deduced peptide mimic loci in *M. hapla*. To dissect the biological activity of the nematode encoded mimics, we developed an assay based on exposing growing seedlings to synthetic peptides and scoring features of root development and/or RKN infection. These experiments demonstrated that RKN peptides induce a dose dependent root phenocopy of native peptide application and over-expression, indicating physiological relevance. Biochemical analysis of the signaling events and specific activity of RKN peptide hormone mimics is in progress. To

shed light on the origin of these genes we mapped the location of the *M. hapla* CEPs to clusters in otherwise gene pauce regions. Intriguingly these regions are highly variable between isolates, possibly indicating evolutionary novelty and roles in host-specificity.

GENOMIC ANALYSIS OF *STEINERNEMA*: INFORMING FUNCTIONAL BIOLOGY AND ECOLOGY. **Dillman, Adler R.[1], A. Mortazavi[1], and P. W. Sternberg[1].** [1]Howard Hughes Medical Institute, Division of Biology, California Institute of Technology, Pasadena, California 91125.

The new generation of DNA sequencers have opened up the entire nematode phylum to whole-genome analysis, thus allowing us to explore the evolution of different genera. The genus *Steinernema* comprises over 60 characterized species that are lethal parasites of insects with differing foraging strategies and host ranges. We have sequenced and assembled the genomes and staged transcriptomes (set of expressed mRNAs) of five whole genomes spanning the *Steinernema* genus (*S. carpocapsae, S. scapterisci, S. monticolum, S. glaseri,* and *S. feltiae*) using the Illumina DNA sequencing platform. Steinernematid genomes prove amenable to Illumina sequencing due to their size (~95 Mb) and high G+C content (~45%). The combination of multiple closely related genomes in a non-*Caenorhabidtis* clade and accompanying deeply sequenced transcriptomes allows for powerful comparisons to other genera such as *Caenorhabditis*. In particular, comparisons in expression at defined stages shows significant plasticity of timing across one-to-one orthologous genes in the 5 genomes plus *C. elegans*. Using available ecological and molecular data we explore genomic differences likely to be involved in host range and specificity. We also examine the utility of these five genomes by orthology analysis within Nematoda, assessing the conservation of biological pathways, analyzing regulatory regions, and evaluating the established relationships within *Steinernema*.

IN PLANTA RNAi SILENCING OF EFFECTOR GENE *16D10* REDUCES PARASITIC SUCCESS OF *MELOIDOGYNE CHITWOODI*. **Dinh[1], Phuong T.Y., C. Bates[1], E.L. Davis[2], R.S. Hussey[3], and A.A. Elling[1].** [1]Department of Plant Pathology, Washington State University, Pullman, WA 99164, [2]Department of Plant Pathology, North Carolina State University, Raleigh, NC 27607, [3]Department of Plant Pathology, University of Georgia, Athens, GA 30602.

The Columbia root-knot nematode (*Meloidogyne chitwoodi*) is a devastating pathogen of potato in the Pacific Northwest of the United States. In potato, *M. chitwoodi* generally does not cause significant quantitative yield losses, but as a quarantine pathogen it can render entire fields unmarketable even when detected at only very low population levels. *M. chitwoodi* infection leads to gall formation on the tuber surface, which reduces the quality of the crop. Even though a *M. chitwoodi* resistance gene has been found in *Solanum bulbocastanum*, a wild relative of cultivated potato (*Solanum tuberosum*), traditional breeding programs have not yet developed potato cultivars that are resistant to *M. chitwoodi*. *Meloidogyne* spp. secrete the products of effector genes from their esophageal gland cells into host plant tissue. The secreted products of effector genes modulate the molecular interactions between the nematode and its host. Previous studies showed that the putative effector gene *16D10* is strongly conserved among temperate and tropical *Meloidogyne* spp. and that its product interacts with SCARECROW-like plant transcription factors *in vitro* and *in planta*. Furthermore, previous analyses showed that RNA interference (RNAi)-mediated silencing of *16D10* suppressed *M. arenaria*, *M. hapla, M. incognita* and *M. javanica* gall formation in *Arabidopsis* by 63-90% and their reproduction by 69-93% compared to control plants. We found that the same *16D10* double-stranded RNA (dsRNA)-generating RNAi construct suppresses gall formation of *M. chitwoodi* by 83% and reproduction (eggs per gram root) by 80% compared to wild type plants when expressed in *Arabidopsis*. Assays to test *16D10*-RNAi-mediated resistance in potato are ongoing. Taken together, our results show that RNAi-mediated down-regulation of effector genes could be a promising new tool to control *M. chitwoodi*.

GROWER ACCEPTANCE OF ENTOMOPATHOGENIC NEMATODES IN FLORIDA. **Duncan, L.W.** Citrus Research and Education Center, University of Florida, 700 Experiment Station Rd, Lake Alfred FL 33850 (USA).

Entomopathogenic nematodes have been widely employed for two decades to manage insect pests in Florida's turf, pasture and citrus industries. Grower adoption of this form of biological control in both low and high value crops is predicated on a variety of factors. The use of *Steinernema scapterisci* to control mole crickets in both turf and pasture represents a unique case of classical biological control using EPNs. The nematode was discovered and introduced to Florida in the 1980s from the center of origin of *Scapteriscus* mole crickets in South America. The propensity of *S. scapterisci* to infect mole crickets and other orthopterans allows it to infect gregarious subterranean hosts that then disperse the nematode widely through flight. As a consequence, and unlike numerous other introduced EPN species, *S. scapterisci* has persisted at high levels in Florida where it is readily found many miles from the locations where it was released. The abundance of mole crickets declined dramatically, coincident with

the introduction of *S. scapterisci* and two parasitoid species. The adoption of this management tactic by many farmers and golf course managers was inevitable, given the effectiveness and long term pest suppression afforded by *S. scapterisci*. In contrast to classical biological control systems, factors affecting the adoption of augmentation (i.e., inundative) biological control are more complex and dynamic. The *Diaprepes* root weevil (DRW) is an invasive pest that became a major threat to citriculture in Florida when organochlorine pesticides were deregistered for agricultural use, due in part to their long residual effects. Because DRW eggs fall to the soil from the tree canopy during all but the coldest months, the short lived pesticides available today provide ample windows of opportunity for neonate larval recruitment into soil and teneral adult emergence from soil. The inability of chemical pesticides to break the DRW life cycle provided an opportunity to develop tactics that employ EPN. Nevertheless, grower acceptance of EPN use faced many constraints, including variable quality control of commercial products and convincing evidence of efficacy. A key element in overcoming these constraints was the establishment of a federally funded task force to study DRW, comprised of growers and state and federal scientists. Research implemented by task force members for nearly a decade provided much of the basic and applied information about the weevil and its natural enemies needed to make rational pest management decisions. Moreover, direct involvement by growers in the task force decision making and research projects ensured a high level of visibility for subsequent management recommendations.

DO BACTERIAL ECTOPARASITES MODULATE THE PREDATION RATE OF ENTOMOPATHOGENIC NEMATODES IN NATURE? **Duncan, L.W.[1], R. Campos-Herrera[1,2], F. E. El-Borai[1,3].** [1]Citrus Research and Education Center, University of Florida, 700 Experiment Station Rd, Lake Alfred FL 33850 (USA). [2]Instituto de Ciencias Agrarias, CSIC, Serrano 115 dpdo Madrid, 28006 (Spain). [3]Plant Protection Department, Faculty of Agriculture, Zagazig University (Egypt).

Independent lineages of *Paenibacillus* spp. appear to have converged as ectoparasites of entomopathogenic nematodes (EPNs) in the genera *Steinernema* and *Heterorhabditis*. In Florida, a non-entomopathgenic *Paenibacillus* sp., closely related to the entomopathogens *P. lentimorbus* and *P. popilliae,* is commonly observed adhering to the cuticle of *S. diaprepesi* infective juveniles that emerge from insect prey. The bacterium is phoretic on and specific to *S. diaprepesi*. It completes its life cycle within insects killed by *Xenorhabdus doucetiae,* the entomopathogenic bacterial symbiont of *S. diaprepesi*. The presence of *Paenibacillus* sp. does not affect the development rate or population size of *S. diaprepesi* in its insect host. However, steinernematid and heterorhabditid nematodes encumbered by *Paenibacillus* endospores migrated less and killed fewer insects in controlled experiments. Whether these bacteria regulate entomopathogenic nematode populations in nature is unknown. We employ real-time quantitative PCR (qPCR) in ongoing field experiments and surveys to study relationships between EPNs and potential competitors and antagonists, including *Paenibacillus* sp., in citrus orchard soils. Positive relationships between the abundance of *Paenibacillus* sp. and *S. diaprepesi* in nematode samples from geospatial ($r=0.38$, $n=54$, $P<0.01$) and temporal ($r=0.36$, $n=432$, $P<0.0001$) surveys support the likelihood of species specificity, both for the primer/probe designs and the bacterium-nematode relationship. Moreover, the temporal patterns of *Paenibacillus* sp. in two orchards on Florida's central ridge were consistent with the possibility that the bacteria may regulate numbers of *S. diaprepesi*. We detected fewer *Paenibacillus* sp. ($P<0.05$) and more *S. diaprepesi* ($P<0.05$) in plots of citrus trees managed conventionally (weekly, microjet irrigation; dry fertilizer) compared to those of trees under intensive management (daily, drip fertigation). Major differences in the soil environments of the two treatments (plant nutrients, pH, EC, soil moisture) provide factors that potentially regulate the bacterium and might be used to measure the role of *Paenibacillus* sp. in EPN population dynamics and perhaps modify soils in ways that favor biological control by EPNs.

EFFECT OF SMALL LIPOPHILIC MOLECULES OF TOMATO AND RICE ON THE BEHAVIOUR OF ROOT-KNOT NEMATODES. **Dutta, Tushar K.[1,2], S.J. Powers[2], M. Birkett[2], H.S. Gaur[1*], and R.H.C. Curtis[2].** [1]Indian Agricultural Research Institute, New Delhi-110 012, India and [2]Rothamsted Research, Harpenden, Hertfordshire, AL5 2JQ, UK. Corresponding Authors: hsg_nema@iari.res.in and rosane.curtis@bbsrc.ac.uk

Plant chemicals in the rhizosphere originating from root exudates, or sites of previous nematode penetration, can influence nematode behaviour and a number of plant compounds, some present in root exudates, have been shown to either attract nematodes to the roots or result in repellence, motility inhibition or even death. The present work was conducted to isolate small lipophilic molecules (SLMs) emitted by root exudates of *Solanum lycopersicum* and *Oryza sativa* to investigate their effect on root-knot nematode. SLMs was extracted through solid phase extraction (SPE) from hydroponically collected root exudates of 40 tomato and rice plants. SLMs had an inhibitory impact on the motility of J2 of *Meloidogyne incognita* and *M. graminicola* and showed a nematotoxic, or nematostatic (upon dilution) effect on both species of RKN. The semiochemicals present in the SLMs did not stimulate nematode stylet thrusting or production of secretions; however, they negatively influenced behavior of

these RKN by affecting their mobility. Therefore, it is proposed that SLMs present in both tomato and rice root exudates play important roles during the interaction of RKN with their host plant, and that they might exert a repellent, or allellopathic effect on these nematodes.

MEMOIRS OF N. A. COBB ON THE OCCAISON OF THE 50TH ANNUAL MEETING OF THE SOCIETY OF NEMATOLOGISTS. **J. D. Eisenback[1].** [1]Dept. of Plant Pathlogy, Physiology and Weed Science, Virginia Tech, Blacksburg, VA 24061.

It is with great pleasure and honor that I address my fellow members of the Society of Nematologists on this celebration of our 50th annual meeting. I am very pleased to see with my own eyes that this society is vibrant and prosperous, and I wish that it will continue to increase in membership and influence during the next fifty years! Since I am considered to be the father of nematology in the United States, I have been asked to make a few remarks about my foray into the science of nematology and leave behind some encouraging words for the future. Although I had a keen interest in books, later I realized that I was very fortunate to have an intimate exposure to a great variety of practical operations. These responsibilities taught me many valuable manual skills, how things should be done, and what it meant to actually do them. For the rest of my life I was able to take my ideas and inventions and to turn them into material forms as devices, instruments, and buildings. These have included cameras and microscopes, darkrooms, laboratories, and numerous other devices. I have used these instruments to solve complex problems by breaking them down into their simpler parts. As scientists we should play a leadership role in simplifying life. In this regard, I am sad to see that the metric system remains to be adopted in the United States, but I am very happy to see that English has become the standard language of science. In the future, I encourage the Society to remain enthusiastic about the science of nematology and to be full of enthusiasm for our little friends, the nemas!

THE EFFECT OF BIOCHAR ON THE REPRODUCTION OF SOUTHERN ROOT-KNOT NEMATODE ON TOMATO. **J. D. Eisenback[1], L. Grainey[2], and D. J. Dodge[1].** [1]Dept. Plant Pathology, Physiology and Weed Science, Virginia Tech, Blacksburg, VA 24061, [2]Plant Disease & Insect Diagnostician, Bartlett Tree Research Laboratory, 13768 Hamilton Road, Charlotte, NC 28278.

Biochar is currently touted as a soil enhancement additive that improves plant growth quality by balancing moisture levels, improving the permeability of clay soils; increasing the cation exchange capacity of soils; buffering the soluble organic carbon; enhancing soil microbe interactions; detoxifying poisons in soil; and increasing plant resistance to fungi, insects, and plant-parasitic nematodes. In addition, Biochar acts as a carbon-sequestering agent in soil that relieves stresses on atmospheric gasses caused by the production of excessive $CO_2$. The purpose of this study was to evaluate the effect that Biochar has on the reproduction of southern root-knot nematode, *Meloidogyne incognita* race 3, on tomato, *Solanum lycopersicon* cv. 'Rutgers'. Five replicates of 4 week-old tomato seedlings were transplanted into 15cm diam. clay pots filled with topsoil, or topsoil amended by volume with 5%, 25%, or 50% biochar, inoculated with 5,000 eggs per pot, and allowed to grow in the greenhouse for 65 days at 22-26°C. Comparisons of the means of the number of eggs per gram of root for each treatment revealed that they were not statistically significant from the control. Likewise, Biochar had no effect on plant shoot length or root weight.

THE EFFECT OF *BRASSICA* AND *RAPHANUS* COVER CROPS ON NEMATODE ASSEMBLAGES IN A POTATO-BASED CROPPING SYSTEM IN SOUTH AFRICA. **Emil Engelbrecht[1], Driekie Fourie[1] and Mieke Daneel[2].** [1]School of Environmental Sciences and Development, Plant Protection, North West University, Private Bag X6001, Potchefstroom, 2520, South Africa, [2]Agricultural Research Council-Institute of Tropical and Subtropical Crops, Private Bag X11208, Nelspruit, South Africa.

Cover crops are used worldwide to manage plant-parasitic nematodes, including *Meloidogyne* spp., in agricultural and horticultural cropping systems. Locally, potato producers in the Western Free State Province experience substantial quality losses in potato due to root-knot nematode infection. Therefore, *Brassica* and *Raphanus* varieties were evaluated to establish their effect on a high root-knot nematode infestation of approximately 38 000 eggs and J2/50g roots of a preceding potato crop that was planted during the 2010 growing season. A field trial was subsequently conducted on this site during the 2010/2011 season. The trial consisted of six treatments, *viz.* two *Brassica* varieties (Nemat and Caliente), two *Raphanus* varieties (Terranova and Doeblet), an untreated control as well as a standard nematicide treatment (EDB@40ℓ/ha). The trial layout represented a randomised complete block design with five replicates for every treatment. Soil samples for nematode extraction were taken prior to planting and during flowering of the cover crops as well as after incorporation of the aerial parts of these crops into the soil. Soil, root and tuber samples of the potato crop planted after these cover crops were also sampled during tuber initiation. In addition, the nutrient status of the soil as well as the organic material content was monitored prior to planting of the cover crops as well as thereafter. Results obtained during flowering of the cover crops showed that root-knot nematodes were maintained in roots/tubers of all the cover crop spp. used in this study. Egg and J2 numbers/50g roots

ranged between 338 for Nemat and 26 553 for Caliente. During potato tuber initiation, egg and J2 numbers/50g potato roots did not differ significantly for the untreated control and the respective cover crop treatments that preceded the potato crop. The EDB treatment, however, resulted in the lowest number of root-knot nematodes/50g roots (4 165), which were significantly lower than those for the untreated control and cover crop treatments.

MARINE NEMATODES FROM BRAZIL, WITH FOCUS ON THE ENVIRONMENTAL HETEROGENEITY CAMPOS BASIN. **Esteves, André Morgado.** Laboratório de Meiofauna. Departamento de Zoologia. CCB. UFPE. Av. Professor Moraes Rego, S/N - Cidade Universitária, Recife - Pernambuco – Brasil. CEP: 50670-420.

1) Past, present and future of marine nematology in Brazil

The visit of Dr. Sebastian Gerlach, in the middle of century XX, was a start point to the study of marine nematodes in Brazil. This researcher had done a taxonomic survey along different sites of the Brazilian coast describing one hundred of new *taxa* (genera/ species). After that, nothing more was done until the beginning of the century XXI when Brazilian's researchers began to participate in international projects, like DARWIN INITIATIVE and the Postgraduate International Nematology Course hold in Ghent, Belgium. It gave rise to a new generation of marine nematologists in Brazil. Meanwhile, relevant environmental programs in the Brasilizian coast started emphasizing the meiofauna, more particularly the marine nematodes. In this context, the Meiofauna Laboratory from Zoology Department of UFPE was pioneer in the taxonomical study of nematodes. Currently, this group is spreading its knowledge and training new groups for the study of marine nematodes in other universities from North-Northeast of Brazil (UFBA, UFAL, UFCG, UFPA) as part of a specific project called Brazilian Marine Biodiversity: Development of the Taxonomy of Marine Nematodes. Besides, the UFPE group is helping other groups at the southeast of Brazil, such as USU and USP. These actions represent the return of the study of marine nematodes in Brazil as well as increase the knowledge of marine nematode biodiversity. These observations can be easily quantified through a simple search at Nemys database (http://nemys.ugent.be). In 2005 and 2006, 21% of the published nematode descriptions of new *taxa* (species/genera) had Brazilian researchers as authors. In the subsequent three years, other 10 descriptions were published by Brazilian nematologists. Nevertheless, to keep increasing our taxonomical efforts at medium and long-term, it is needed to maintain the financial support for further studies, especially training young researchers as well as exchanging experiences between current Brazilian and foreign researchers. This latter contact will keep us continuously updated about new techniques applied and the availability of new equipments.

2) Marine nematodes from Campos Basin

The Phylum Nematoda is the most abundant group in several environments. In Campos Basin, nematodes are used for ecological and taxonomic studies in continental margins, including submarine canyons. The aim of this work is to characterize the nematofauna from Campos Basin, identifying the relations between the main genera and the two studied environments (continental shelf and slope) in two different seasons (winter 2008 and summer 2009). In total, nine profiles and eleven isobaths were prospected being five isobaths at the continental shelf (25m, 50m, 75m, 100m and 150m) and six at the continental slope (400m, 700m, 1300m, 1000m, 1900 m and 2500m). Samples were taken using a Box corer modified to sample 0,25m$^2$ of the sediment. At each station, three replicate were prospected. The nematofauna from the two environments were composed of approximately 300 genera. *Sabatieria* is the most abundant genus in nearly all stations of both environments and seasons. The nematode composition, richness and the correlations between nematodes and sedimentological variables are totally unknown for the Brazilian continental margin. Therefore, these results will contribute to the knowledge of the Brazilian biodiversity in deep sea as well as to the increase of the biodiversity of this ecosystem as a total.

ORGANIC AGRICULTURE AND COCOA PRODUCTION: PROSPECTS AND IMPLICATIONS FOR CONTROL OF SOIL-BORNE PATHOGENS (EMPHASIS ON NEMATODES). **Fademi, O. A[1]., M. O. Ogunlade[1], S. O. Akanbi[1], S. B. Orisajo[1], B. O. Obatolu[1], M. A. Jolaoso[2] and G. O. Iremiren[1].** [1]Cocoa Research Institute of Nigeria, Km 14, Ibadan/Ijebu-Ode Rd., Idi-Ayunre, P. M. B. 5244, Ibadan, Nigeria; OR 97331, [2]Raw Materials Researcg and Development Council, Plot 427 Aguiyi Ironsi Street, Maitama District, P. M. B. 232, Abuja, Nigeria.

In Nigeria more than 75% of the cocoa growing soil is infested with nematodes predominant among which are *Meloidogyne incognita*, *Helicotylenchus* spp., *Radopholus* spp., and *Xiphinema* spp. had about 55% spread. Although it is more than 50 years since cocoa gain prominence in Nigerian agriculture being the driving force for the economy before oil boom, research efforts on nematode control revolved around use of chemicals up till some ten years ago. Although losses from nematode infestation especially at establishment stage range from 50-75%, growers rarely resort to using nematicides due largely to low perception of the problems.

Nigerian nematologists did not show interest in biological control of nematodes in tree crops in particular until the past 10 – 15 years. Limited progress has however, since been made towards the development of practical system of use of organic amendment (OA) for control of nematodes in field and tree crops. The reason for this slow progress include lack of knowledge of the ecological relations that occur within the rhizosphere on application of

OA and in lack of data on the population dynamics of nematodes in a continual application field and lack of commitment to determining how OA can be mass produced and packaged for delivery to soil in a viable state. An analysis of the recently published literatures suggests that much of the current work in OA does not address these issues. It is suggested that successful development of OA will require a redirection of resources towards OA, a commitment by researchers to extend this work from laboratory into the field, a general improvement in the quality of OA research, a concentration in key issues and more commercial involvement.

ADVANCES IN NEMAPLEX, AN ON-LINE INFORMATION AND MANAGEMENT RESOURCE FOR PLANT AND SOIL NEMATODES. **Ferris[1], Howard.** [1]Department of Nematology, University of California, Davis, CA 95616, USA.

Initiated in the 1980s as a nematode identification tool for use in a class on the biology and management of plant-parasitic nematodes, Nemaplex (http://plpnemweb.ucdavis.edu/nemaplex) has evolved with the development of the personal computer and evolution of the abilities of its developer. Early features included pages on nematode biology, ecology, host-parasite relations, management, history, personalities, and lecture outlines, laboratory schedules and other class materials. Through the 1990s, reflecting interests and activities of the developer's laboratory, Nemaplex expanded into the biology and ecology of bacterivore fungivore, predator, and some animal-parasitic nematodes. During the past decade, web-based delivery and accessibility of management tools and exercises have been incorporated, including the background rationale and procedures for analyzing soil faunal structure and function. Recently, searchable databases have been integrated into Nemaplex. They provide data on the host status of plants to nematodes and the selection of resistant cultivars and appropriate rotation crops and cover crops. The databases also provide ecophysiological parameters of soil nematodes, including their carbon utilization and metabolic footprints. Other recent features include extensive keys in support of nematode identification, a dictionary of nematological terminology, and sources of information on nematicides. Nemaplex currently consists of over 7,000 web-page files and 4,400 pictures which are connected through 50,000 hyperlinks to allow review and analysis of a wide range of topics. The framework allows for instant update as new information becomes available and time permits. Some of the developments in Nemaplex have arisen in response to user inquiries, so user comments and suggestions are appreciated.

CURRENT AND FUTURE DIRECTIONS IN NEMATODE FAUNAL ANALYSIS. **Ferris[1], Howard.** [1]Department of Nematology, University of California, Davis, CA 95616, USA.

Carbon and energy determine the size and activity of the soil foodweb. They fuel the contributions of nematodes and other soil organisms to ecosystem services and disservices. The rates of depletion of resources in the metabolic and growth processes of successive consumers affect their availability to higher trophic level organisms. Indices based on relative proportions of nematode functional guilds are bioindicators of the framework of ecosystem structure and of its potential for providing services. The magnitudes of services and disservices depend on the biomass and activity of the organisms present. The challenge in sustainable systems is to manage the disservices of soil nematodes within the context of stewardship of their beneficial services. Estimates of carbon utilization by various functional guilds of nematodes provide metrics of their contributions to services. The regulation of population levels of opportunistic herbivore nematode species, as an example, is the integral function of many organisms, including predaceous and omnivorous nematodes. Management to ameliorate nematode disservices often results in unintended, but long-lasting, collateral disruption of these higher trophic level organisms. Effective pest regulation requires co-location of predator and prey organisms, or overlaps in their ranges, and may be measured as connectance within the system. Understanding of the mechanisms of suppressive and regulatory effects will be enhanced by application of functional genomics and molecular tools to unravel evolutionary and ecological processes within the immense diversities of form, function, micro-niches and spatial scales that characterize organisms of the soil ecosystem. Belowground biodiversity and food web connectance can be enhanced by resource subsidy to increase organism abundance, and by mitigation of environmental constraints to organism survival and function. Diversity and prevalence within guilds of organisms ensures functional continuity through time and among spatially separate micro-niches. Advances will require continued integration of ecology and molecular biology, including the linkage of barcoding and functional genomic fingerprinting with attributes of organisms and the experimental validation of the evolving concepts of soil ecosystem interactions and dynamics.

THE WORM THAT TURNED: BACTERIAL SYMBIONTS OF ENTOMOPATHOGENIC NEMATODES AS A POTENT SOURCE OF NOVEL BACTERIAL TOXINS. **ffrench-Constant, Richard H., P. Wilkinson and A. J. Dowling.** Biosciences, University of Exeter, Tremough, Penryn TR10 9EZ, UK.

We are looking at *Photorhabdus* and *Xenorhabdus* nematode symbionts as a potent new source of insecticidal toxins. Recently this work has involved the whole genome sequencing of key strains of these entomopathogenic

bacteria and the design of novel high-throughput screens to look at their effects on other microbes and invertebrates. We will discuss the results of these screens and examine the mode of action and potential utility of the novel insecticidal toxins discovered, specifically, the Toxin complexes or 'Tc's' and the Makes Caterpillars Floppy toxins 1 and 2 or 'Mcf1 and Mcf2'. We will also discuss the recent sample sequencing of a *P. asymbiotica* strain from Australia and discuss how the recent acquisition of plasmids may have facilitated its ability to infect man.

DIFFERENTIAL EXPRESSION OF BETA1,4-ENDOGLUCANASE INDUCED BY DIET CHANGES OF FOLIAR NEMATODE *APHELENCHOIDES FRAGARIAE*. **Fu, Zhen and P. Agudelo.** School of Agricultural, Forest, and Environmental Sciences. 114 Long Hall, Clemson University, Clemson, SC 29634.

The foliar nematode *Aphelenchoides fragariae* is an economically important parasite of a wide range of plants. It can feed endo- and ectoparasitically on aerial parts of plants, causing characteristic lesions and distortions, but it can also be mycetophagous. For research purposes, foliar nematodes are often maintained in pure cultures on fungi. The effect of the diet (plants vs. fungi) on pathogenicity and/or virulence of this nematode has not been studied. Our objective was to follow differences in expression of a ß-1,4-endoglucanase induced by changes in nematode diet. We identified and sequenced an endoglucanase from *A. fragariae* using degenerate primers designed from publicly available sequences from other plant-parasitic nematodes. We then designed specific primers for a portion of the selected gene (*Af-eng*) and measured expression levels on a population of foliar nematodes maintained on a fungus (*Cylindrocladium* sp.) and on hosta plants. When changing a population from feeding on the plants to feeding on the fungus, *Af-eng* expression levels decreased 1,800-fold, after approximately five generations. To obtain a measure of selection pressure on the population, we also verified the presence of the gene on individuals. After 5 generations, *Af-eng* is detectable in 75% of the individuals feeding on plants and in 25% of the individuals feeding on the fungus. After more than 50 generations, the gene is no longer detectable in the population feeding on the fungus. In greenhouse studies, using four different hosta cultivars, we observed differences (p=0.05) in the severity of symptoms caused by foliar nematodes maintained on the fungus (leaf area affected ranged from 1.21% to 4.13%) and by those maintained on the plants (leaf area affected ranged from 4.17% to 25.95%). Nematodes maintained on plants for more than 50 generations also had higher reproduction on the hosta cultivars evaluated. Nematode cellulases have been implicated in plant-parasitism of several nematode species, and they have been well-characterized in several tylenchids, although their origin remains controversial. We report the first study of differential expression induced by diet changes of an endoglucanase in *A. fragariae*, and discuss the potential role of the enzyme in the ability of this nematode to cause disease on plants.

PHENOTYPIC DIFFERENCES BETWEEN *MELOIDOGYNE HAPLA* STRAINS AND GENETIC APPROACH TOWARD IDENTIFICATION OF THE RESPONSIBLE GENES **Fudali, Sylwia L., J. Gimeno, V.M. Williamson,** Dept. of Nematology, University of California, Davis, CA 95616.

Root knot nematodes (RKN) are sedentary endoparasites of many economically important crops. They can induce specialized feeding sites composed of enlarged, metabolically active giant cells inside the roots of a wide range of host plants. We use the Northern root knot nematode, *Meloidogyne hapla*, as a model to identify genes that affect the parasitism phenotype. *M. hapla* was selected for this study because it reproduces by facultative meiotic parthenogenesis enabling both self crossing and outcrossing, which makes genetic studies feasible. Its 54Mbp genome has been sequenced and annotated and a genetic map has been produced based on analysis of molecular marker segregation in F2 lines from a cross between two *M. hapla* strains VW8 and VW9. Our current project focuses on traits that differ between *M. hapla* strains VW9 and LM. These parental strains were obtained from geographically different locations and differ in a wide range of phenotypic traits. VW9 and LM differ in their attraction to certain chemicals and to roots of specific hosts. Moreover, LM is virulent on nematode resistant common bean cultivar NemaSnap, while VW9 is not able to reproduce on this host. The parental strains also differ in ability to parasitize and reproduce on a model plant *Medicago truncatula*. LM strain is more attracted to *Medicago* roots and induces bigger galls than VW9 strain. In contrast, VW9 produces more egg masses with more eggs than LM on *Medicago* roots. As a result of crossing VW9 and LM, we have obtained a mapping population consisting of 120 F2 lines. SNP polymorphisms will be characterized in these lines to produce a molecular linkage map. In parallel, we will test F2 lines for phenotypic traits including attraction to specific chemicals and hosts and reproduction on resistant NemaSnap bean. Comparison of the segregation of molecular and phenotypic traits will allow us to map and to identify candidate genes that contribute to the corresponding traits. Functional analysis of these candidates will be carried out to identify genes that contribute to differences in ability to parasitize specific hosts.

NEW ADVANCES IN EPNS AROUND THE WORLD. **Ganguly[1], Sudershan and C. Dolinski[2].** [1]EPN Genomics Lab, Division of Nematology, Indian agricultural Research Institute, New Delhi-110012, India, [2]Universidade Estadual do Norte Fluminense Darcy Ribeiro/CCTA/LEF, Av. Alberto Lamego, 2000, Pq. California, Campos dos Goytacazes, RJ, Brazil, 28015-602.

Research on Entomopathogenic nematodes (EPNs) and their bacterial symbionts is being pursued by several researchers in different parts of the world. This paper gives an overview of advancements in this area mainly in Asia and South America. In South America, the research on EPNs and their application as biocontrol agents started during 80's with timid lab bioassays focusing in different soil pests. A new era for EPNs in South America started with a workshop in São Paulo, Brazil (2000). Then in 2001 there was a National Course of Biological Control of Pests with EPNs in Campos dos Goytacazes, Brazil, and in 2003 a Latin American Symposium on Entomopathogenic Fungi and Nematodes. Nowadays, surveys and pathogenicity tests are being carried out in Brazil, Chile, Venezuela and Colombia, while studies on mass production, storing and formulation are being done in Brazil and Colombia. Other studies dealing with taxonomy, biology and classical genetics are also being carried out by different groups in Brazil. In Asia, EPN biodiversity have been explored, and their bioefficacy against more than 40 species of insects belonging to Lepidoptera, Diptera and Coleoptera, infesting vegetables, field crops, fruit trees, turf grass, mushrooms and forest trees, had been established in China, India, Japan and Korea. Efforts have also been on the comparative efficacy of different EPN species and their $LC_{50}$ dose against major local insect pests in respective countries. Out of 88 known species of EPNs (*Steinernema*:70 and *Heterorhabditis*:18), 37 species have been described from Asia, the maximum from China (14), followed by Vietnam (10), two each from India, Japan. Pakistan and Thailand, and one each from Indonesia, Korea, Nepal, Oman and Turkey. Although several strains of EPNs have been isolated from China, India, Japan and Vietnam, and their sequences of ITS region of rDNA have been deposited in NCBI GenBank, yet most of these isolates are still in queue waiting to be identified. Information has also been generated on the ecological characterisation of EPNs and their symbionts, application technology, mass production, efficacy of bacterial metabolites against insects as well as fungal pathogens (*Phytophthora infestans*, *Rhizoctonia solani*, *Sclerotium* sp.) but there has been limited efforts on the formulations and commercialisation aspects. Nevertheless, a few formulations, for eg. *S. kushidai* -based 'Shibaichi-Nema' and *S. glaseri* -based 'Biotopia' in Japan, and *S. thermophilum* -based 'Pusa NemaGel' in India, and *S. carpocapsae* -based 'Sesil' in Korea, have been developed and commercialised. All these advancements are aimed to effectively utilise these nematodes against soil and foliar pests of agricultural importance. There is an urgent need to pursue EPN research in consortium mode wherein nematologists, entomologists, microbiologists and chemists should work hand-in-hand to explore the vivid aspects of EPNs along with their bacterial symbionts and the insect hosts (ENBI complexes), for exploiting their maximum biocontrol potential for agricultural sustainability.

ADVANCEMENTS IN ENTOMOPATHOGENIC NEMATODES-BACTERIA- INSECT (ENBI) COMPLEXES IN INDIAN SUB-CONTINENT. **Ganguly, Sudershan, S. Kumar and K.S. Rathour.** EPN Genomics Lab, Division of Nematology**,** Indian agricultural Research Institute, New Delhi-110012, India**;** Email:sg_nema@yahoo.com

The increasing awareness about the environmental and health hazards caused by chemical pesticides and the demand for organically produced agricultural products in the international trade, have drawn the attention of plant protection scientists towards naturally occurring bioagents. Entomopathogenic nematodes (EPN) belonging to the families Steinernematidae and Heterorhabditidae, have tremendous biocontrol potential against several household as well as agricultural insect pests. EPN research in India initiated in 1966 and till 1987, much of the work concentrated on the efficacy of exotic strains against local insect pests of rice, sugarcane and other field crops. Due to the poor adaptability of these strains under Indian conditions, the results on field efficacy were not consistent and therefore, a need to search for indigenous strains of EPNs was felt. Resultantly, several strains were isolated, leading to the descriptions of Heterorhabditis indica Poinar et al., 1992 from Tamil Nadu; Steinernema thermophilum Ganguly & Singh, 2000 from New Delhi and identification of S. abbasi, S. bicornutum, S. carpocapsae, S. feltiae, S. glaseri, S. riobrave, S. tami, S. siamkayai and H. bacteriophora from local soils. Organized research on EPNs is being pursued at I.A.R.I, New Delhi; G.A.U, Anand and N.B.A.I.I, Bangalore. The heat tolerant species S. thermophilum Ganguly and Singh, 2000, is the first species of this genus from Indian soil. It was characterized ecologically for knowing its optimum temperature and moisture requirements, foraging behaviour and host range, which indicated that this species would be an ideal bioagent for managing a wide range of insect pests of crops not only in India but also other tropical and subtropical regions of the world. Its field efficacy was demonstrated at farmer's field against Plutella xylostella, Spodoptera litura, Chilo partellus and subterranean termites on cabbage, cotton, maize and wheat, respectively. S. thermophilum has been characterized morphologically using SEM, biochemically, and the base sequence of its entire ITS region of rDNA has also been submitted to the GenBank. Its symbiotic bacterium has been identified and described as new species

Xenorhabdus indica. Remarkable achievement has been made in developing its novel biopesticidal formulation namely Pusa NemaGel with enhanced shelf-life at low as well as high temperature conditions. In 2006, a patent application for this formulation was filed and the bench-scale formulation technology, has been assigned to NRDC for commercialization. The bioagent S. thermophilum has also been licensed to Multiplex Biotech International Ltd., which is available in the market for its widespread application. During 2004-2011, several novel isolates of EPNs have been explored and characterized the rhizosphere soil of different cropping systems in almost all the states of India, representing 11 agro-climatic regions varying from hot arid (rainfall < 40 cm), semi-arid, temperate to tropical hilly regions with high annual rainfall (>400 cm), through various projects funded by Indian Council of Agricultural Research (ICAR), Department of Science and Technology (DST), National Research Development Corporation (NRDC) and World Bank. Altogether 26 isolates of Steinernema and 5 of Heterorhabditis were collected. These were characterized based on their isozymic profiles (esterase, catalase, SOD and MDH), RFLPs, sequence as well as composition of ITS region of ribosomal DNA of their infective juveniles. All the sequences were submitted in the NCBI GenBank. The isolates and their respective GenBank accession numbers are: S. thermophilum (DQ66565), Steinernema species 25 isolates viz., SGdl1(EF216870), SGdl2 (EF216871), SGgj1 (GQ373382), SGchh1 (GQ438788), SGmg1 (EF219458), SGut1 (GQ 353372), SGas1 (FJ715946), SGwb2 (FJ418045), Sgwb5 (HQ317503), SGrj16 (GU354214), SGrj17 (HQ317504), SGrj18 (HQ317505), SGrj4 (HQ003711), SGrj5 (HQ003712), SGrj19(GU354215), SG rj20 (HQ148711), SGmtr10(GU354213), SGmtr11 (GU35421), SGmtr12 (GU354216), SGkr (FJ715947), SGjk (FJ418046), Gup1(GQ373381), SGor1(GQ353373), SGtn1(HQ317501), SGtn2(HQ317502) and Heterorhabditis species 5 isolates viz., SGmtr15 (GU354219), SGmtr14(GU354218), SGmtr13 (GU354217), SGmg3 (FJ751864) and SGgj (FJ744544). A dendrogram was constructed by hierarchical cluster analysis based on these sequences and the known species of Steinernema, exhibited trichotomy. Four isolates of Steinernema, one isolate each from Uttaranchal (SGut1) Kerala (SGkr), Rajasthan (SGrj20) and Assam (SGas1) states having paired horn-like structures on their lip region formed a clade along with other 5 species of the bicornutum group, which showed the robustness of this molecular tool in taxonomy. Most of the native isolates of Steinernema formed clusters either within the carpocapsae group or siamkayai group. All the Heterorhabditis isolates were grouped in distinctive one clade. It indicated that unlike feltiae and glaseri groups, the species with small sized infective juveniles (carpocapsae and siamkayai groups) are highly adaptive to temperate as well as tropical and subtropical climatic conditions prevalent in the Indian subcontinent. The World Bank funded National Agricultural Innovation Project operates in consortium mode wherein Nematologists, Entomologists, Microbiologists and Agricultural chemists are working hand-in-hand to explore the nature of interactions among the EPNs, their bacterial symbionts and the insect hosts (ENBI complexes), the Division of Nematology at IARI being the lead center. The project aims to explore the biodiversity of EPNs and EPBs; their molecular characterization and differentiation; their relative biocontrol potential against the insect pests of national importance; their ecological characterization; their precise role in pathogenesis; virulence mechanism involving cellular, histopathological and biochemical changes and identifying the biotoxins from the secondary metabolites of the bacteria and protein complexes involved in host's septicemia. Bacterial symbionts associated with EPNs were also isolated and characterized based on their isozymic profiles (esterase, SOD and MDH), RFLPs, sequence and base composition of ITS region of rDNA using specific primers by Rainy's et al. GenBank accession numbers are: X. indica (AM040494), Xenorhabdus species 9 isolates viz., Xeno-dl2 (GQ373384), Xeno-gj1 (HM749976), Xeno-ch1 (GQ373383), Xeno-mg1 (GU980746), Xeno-as1 (GQ373385), Xeno-kr1 (GU980747), Xeno-jk1 (GU731205), Xeno-champ (GU731206), Xeno-or1(HM749977). Hierarchical cluster analysis with 19 known species of Xenorhabdus placed 7 isolates one each from Delhi, Kerala, Meghalaya, Chhattisgarh, Gujarat, Assam and Uttaranchal in X.indica group. Isolate from Jammu & Kashmir (GU731205) shares homology with X.poinarii and one isolate from Orissa (HM749977) with X. stockiae. The EPNs alongwith their bacterial symbionts, collected from diverse agro-climatic conditions of India, will prove to be useful bioagents for managing a wide range of insect pests of crops in most of the tropical and sub-tropical regions of the world. These are available at Indian Agricultural Research Institute, New Delhi for commercialization.

MUSTARD SEED MEAL REDUCES VIABILITY AND HATCHING OF *GLOBODERA PALLIDA*. **Gao, Xuebiao[1], W.J. Price[1], C. Bates[2], M.J. Van Sickle[3], J. Worapong[1], J.B. Johnson[1], and R.S. Zemetra[1].** Department of Plant, Soil, and Entomological Sciences, University of Idaho, Moscow, ID 83844, [2] Department of Plant Pathology, Washington State University, Pullman, Washington 99164, and [3]Idaho State Department of Agriculture, Twin Falls, ID 83303.

Mustard is a well-known plant with biocidal activities. Mustard seed meals release volatile molecules, which show promise as alternatives to traditional fumigation compounds of plant-parasitic nematodes. We assessed the efficacy of seed meal from oriental mustard (*Brassica juncea*) to reduce the viability of the pale cyst nematode (PCN), *Globodera pallida* which is a destructive plant-parasitic nematode of potato. Seed meal at the dosages of 0.5, 1 and

2 tons/ha was mixed with autoclaved sand. Fifteen cysts enclosed in a nylon bag, were placed 2.5 cm below the soil surface in a plastic cup containing 100 $cm^3$ of autoclaved sand mixed with mustard seed meal. Thirty milliliters of deionized water were added to each cup to approximately saturate the soil with moisture before it was covered with a lid and sealed. The cysts containing cups were placed in a greenhouse for 1, 2, and 3 week periods. Viability of *G. pallida* was assessed by staining eggs with 0.05% Meldona blue. Hatching percentages of *G. pallida* were determined in potato root diffusate (PRD) produced for 2 months with an improved protocol. The experiment was run twice, and the data of the duplicated trials were pooled for analysis. Among the 4 treatments for the 1 week period of cyst exposure, egg viability of *G. pallida* was 74.0% in the control, whereas egg viability in the treatments with the 3 dosages ranged from 59.9- 62.7%. For the 2 week period of cyst exposure, egg viability of *G. pallida* was 58.6% in the control and 40.1-49.1% among the seed meal treatments. At the 3 week period of cyst exposure, the egg viability was 67.2% in the control, whereas egg viability rates in the treatments were 44.7-50.7%. In addition, egg hatching rate was 10.0% in the controls for the 2 week period of cyst exposure to mustard seed meal, while the egg hatching rates in the 3 seed meal treatments ranged from 4.0 - 5.1%. The corresponding egg hatching rate for the 3 week period was 9.1% in the controls and 0.5 - 1.4% for the other treatments. The results indicate that longer exposure time to mustard seed meal leads to higher efficiency in reducing *G. pallida* viability and hatching.

THE IMPACT OF CLIMATE CHANGE ON PLANT-PARASITIC NEMATODES. **Hari S. Gaur,** Indian Agricultural Research Institute, New Delhi-110 012, India. E-mail: hsg_nema@iari.res.in

Climate change expressed as elevated temperature and $CO_2$ level, altered seasonal and rain patterns and extreme events influences agricultural productivity in many direct and indirect ways. Besides abiotic alterations, changes in the physiology of the plant and diversity and activities of the macro- and microfauna and flora also occur. The plant parasitic nematodes, as primary consumers, occupy an important place in food-web and at certain population densities one or more of their species attain the pest status for one or more crops in a particular ecological niche. The niche characteristics get altered with climate change and so the occurrence and dominance of particular nematode species, their competitors, antagonists and associates in a community. There are already reports of changes in geographical distribution of species, and possibilities of establishment of present subtropical and tropical species in regions that are subtemperate or temperate today. Increased temperatures will amount on the one hand, in greater cumulative degree hours that would allow shorter life-cycles and more generations of certain species of nematodes, while on the other hand, increased frequency of extreme low or high temperatures will have sub-lethal or lethal effects that may bring down population densities. Elevated $CO_2$ levels may increase photosynthesis and probably more food availability for the nematodes. The increased infestation and crop damage commonly observed in polyhouses forecast the situation that may occur in open fields in future. With changes in duration of summer and winter months the population dynamics of nematodes will change, requiring readjustments of cropping schedules to escape crop damage due to nematodes and other pests. There is a possibility of change in chemical composition of the food, such as altered C:N ratio, but at the same time host plant tolerance may be altered. The altered ecosystem function, especially decomposition processes, will modify microbial community structure that will have positive or negative impact on nematodes. Nematodes will use survival adaptations to face extreme events including heat and desiccation. The existing nematodes are likely to gradually adapt over generations to the rate of climate change and thus persist. Use of nematodes as model organisms and bioindicators, together with GIS and simulation models may help in climate change research for adaptation and mitigation. Expertise in morphological and molecular taxonomy will be essential to understand changes in community structure. Emergence of heat and desiccation tolerant races, invasive alien species and increased susceptibility of crops to nematodes under stress will be new challenges. At the same time, developing resistant crop varieties and newer methods of nematode management will be new opportunities for applied nematologists. Collaboration between nematologists and agronomists will be required since some of the agronomic practices designed for soil and moisture conservation have been found to aggravate some nematode problems. With increasing emphasis on zero-tillage and conservation agriculture, management of plant parasitic nematodes will be important. Nematodes can also serve as sources of stress tolerance genes that may be useful for crop improvement to meet the impending climatic challenges.

COMMERCIALIZATION OF ENTOMOPATHOGENIC NEMATODES: AN INSDIER'S PERSPECTIVE. **Georgis, Ramon.** Brandt, 2935 S. Koke Mill Rd, Springfield, IL 62711.

Progress in nematode commercialization during 1990's and 2000's was phenomenal. Development in large-scale production technology, quality control, proper label directions and easy-of-use formulations led to the successful commercialization of the nematodes in selected markets. Despite this progress, the reality is that insecticidal

nematodes have not reached their anticipated market potential. This is attributed mainly to competitions from new pesticide and biological chemistries, short shelf life, and lack of proper support from the distributors. The development of nematodes for effective insect control in the context of sustainable agriculture will be a major challenge. A truly integrated approach is required, in which all agriculture practices, including other insect control options, should be considered to obtain maximum effect.

THE AMAZING AFRICAN FIG-NEMATODES OF MARTIN *ET AL.*: FORTY YEARS ON. **Giblin-Davis[1], R. M., N. Kanzaki[1,2], K. A. Davies[3], and B. J. Center[1].** [1]University of Florida-IFAS, Fort Lauderdale Research and Education Center, 3205 College Ave., Davie, FL 33314, [2]Forest Pathology Laboratory, Forestry and Forest Products Research Institute, 1 Matsunosato, Tsukuba, Ibaraki 305-8687 Japan, and [3]Centre for Evolutionary Biology and Biodiversity, School of Agriculture, Food and Wine, The University of Adelaide, Waite Campus, PMB 1, Glen Osmond, South Australia 5064, Australia.

In 1973, Martin *et al.* first reported (Journal of Nematology, 5:77-78) the possibility of a wide variety of unusual tripartite relationships among African figs, fig wasps and nematodes. In 1971, they had surveyed nematodes associated with six African native and seven non-African *Ficus* species in Zimbabwe (Rhodesia) and confirmed that nematodes and wasps were only associated with native figs, and that nematodes had parasitic or phoretic associations with pollinating wasps that facilitated their dispersion to the interior of individual fig sycones. More than 20 species of nematodes (aphelenchs and diplogastrids) were recognized from the survey, but none was identified or described in detail. Fortunately, these materials became available to us as bulk, formalin-fixed vouchers, and some specimens were still in reasonably good condition for identification attempts. We observed *Teratodiplogaster* species in the voucher materials from the figs of the section *Sycomorus* from Zimbabwe, including *Ficus sur* (=*capensis*), *F. sycomorus* and *Ficus* sp. Most were not sufficiently well-preserved for definitive species identification or description, except a series from *Ficus* sp. which is being proposed as a new species to science. It is significantly different from the only other described species, *T. fignewmani,* in the genus *Teratodiplogaster* from *F. racemosa,* of the section *Sycomorus*, from Australia. Similar to what we have observed in Australia in two species from the *Ficus* section *Sycomorus* (*F. racemosa* and *F. variegata*), we identified a *Schistonchus*, a *Mononchoides*, a *Teratodiplogaster* and a species or two of *Parasitodiplogaster* in the fig species from the section *Sycomorus* from Africa. In fact, one species of *Parasitodiplogaster* from *F. sur* was so different that it is requiring a revision of the generic definition. In addition, we observed a very unusual species of diplogastrid (putative new genus) from *F. sur* and a species of *Schistonchus* from *F. burkei* (section *Urostigma*). Almost forty years after Martin *et al.* (1973), we are still discovering amazing details about these interesting associations.

F2 LINES OF *MELOIDOGYNE HAPLA* AS A RESOURCE FOR INTEGRATING THE GENETIC AND SEQUENCE MAP AND FOR IDENTIFICATION OF PATHOGENICITY TRAITS. **Gimeno, Jacinta[1], V.P. Thomas[1], S.L. Fudali[1], J. Schaff[2], D.McK. Bird[3,4], E.H. Scholl[3], D.M. Nielsen[4,5] and V.M. Williamson[1].** [1]Dept. of Nematology, University of California, Davis, CA 95616. [2]Genome Sciences Lab., [3]Dept. of Plant Pathology, [4]Bioinformatics Research Center, [5]Dept. of Genetics NC State University, Raleigh, NC 27695.

The availability of the genome sequence for *Meloidogyne hapla* strain VW9 has propelled its development as a platform for genetic studies and as a model plant parasitic nematode. A cross between *M. hapla* strains VW8 and VW9 permitted to the production of a set of F2 lines and construction of a genetic map based on AFLP markers. These F2 lines are maintained on tomato as resources for genetic studies, map-based cloning, and identification of quantitative trait loci (QTL) involved in parasitism. With the VW9 genome as a reference, sequence of a second strain, VW8, has been obtained and used to identify single nucleotide polymorphisms (SNP). DNA from 182 F2 lines was scored for SNP and sequenced AFLP markers resulting in the positioning of sequence scaffolds, and thus genes of interest, on the genetic map. SNP analysis showed 1:1 segregation of co-dominant molecular markers and a paucity of heterozygotes indicating a novel mechanism of meiotic parthenogenesis. Comparison of genetic distance between SNP with available sequence scaffolds revealed that *Meloiodgyne hapla* has an unusually high recombination rate. This high recombination rate has allowed the fine mapping of a trait required for clumping behavior in nematodes, which segregates as a single locus. In addition, phenotypic analysis of F2 lines showed that attraction of juveniles to the wild potato *Solanum bulbocastanum* as well as gall size and reproductive potential segregate as QTL. Analysis of phenotypes of F2 lines has identified QTL that contribute significantly to each of these phenotypes. A second genetic cross has been carried out between strains LM and VW9 to produce lines that can be used to identify loci segregating for additional pathogenicity traits. Analyses of the segregation of traits in the F2 lines that affect reproductive outputs and attraction to the model plant *Medicago truncatula* are in progress. A cross-species eQTL approach is underway to dissect differences in plant response to infection by the F2 lines and to identify the nematode genes responsible for these differences.

HOW TO MANAGE DAILY STRESSES: THE ENTOMOPATHOGENIC NEMATODE PRESPECTIVE. **Glazer, Itamar.** Nematology, ARO, Volcani Center, 56 Hamakabim Rd. Rishol LeZion, 50-250, Israel.

The entomopathogenic nematodes (EPN) which are used for biological control of insect pests throughout the world are effective only in protected niches. That is due to their sensitivity to environmental stress such as dryness, extreme temperature and UV radiation. Little is known about the biochemical and molecular events underpinning nematodes' physiological responses to stresses. The recent development of Post-genomics research tools may offer an opportunity to understand the stress response better. The presentation focuses on recent progress in understanding the molecular mechanisms of stress response associated with water-loss in EPN and discusses the scope for applying the knowledge and tools for improvement of EPN as biological control agents.

GENES THAT ARE INVOLVED IN THE RECOVERY PROCESS IN THE ENTOMOPATHOGENIC NEMATODE *HETERORHABDITID BACTERIOPHORA*. **Glazer[1], Itamar, A. Moshayov[1] and H. Koltai[2].** [1]Dept. of Nematology, [2]Dept. of Ornamental Horticulture, ARO, Agricultural Research Organization, The Volcani Center, P.O. Box 6, Bet Dagan 50250, Israel.

Recovery in nematodes defines as: exit from developmental arrest and resuming growth and development. In entomopathogenic nematodes this process occurs when the infective juvenile (IJ) enters the insect host, as a response to insect hemolymph. The recovery process is the first outcome of the host-parasitic interaction and is also a commercially very important process. This study was aimed at identifying genes that are putatively involved in this process in *Heterorhabditid bacteriophora*. For this purpose, a large scale bioassay for recovery was established and two subtractions libraries of recovered IJs subtracted by arrested IJs were constructed. Six hundreds expressed sequence tags (ESTs) were sequenced and annotated, resulting in 300 useful ESTs that were compared to *C. elegans* Wormbase and categorized into functional categories according to gene ontology. Of these, twenty three genes were further analyzed. Their expression in the recovery process was determined by quantitative (q) RT-PCR. The expression pattern supported the results obtained from the subtraction libraries. Further analysis of these genes was done by RNAi-based functional analysis in *H. bacteriophora*. Silencing twenty three genes by dsRNAi result in different phenotypes. Bioassay for recovery of IJs harboring different silenced gene was preformed. We found 8 genes that when silenced reduced IJs recovery dramatically compared to recovery in WT IJs of *H. bacteriophora*. Thus these genes are critical in the recovery process. The relation of six of these genes to recovery requires further study. However, the other two genes are connected to insulin/IGFI pathway which is known to regulate dauer formation in *C. elegans*. We will further use a whole transcriptome analysis which will provide a better understanding of the biological pathways and molecular mechanisms of EPNs recovery process.

UNDERSTANDING MICROBIAL SYMBIOSIS USING THE ASSOCIATION BETWEEN *XENORHABDUS* BACTERIA AND *STEINERNEMA* NEMATODES. **Goodrich-Blair, Heidi**[1] and **S. Forst**[2]**.** [1]Department of Bacteriology, University of Wisconsin-Madison, 1550 Linden Dr. Madison, WI, 53726 and [2]Department of Biology, University of Wisconsin-Milwaukee, 3209 N. Maryland Ave, Milwaukee, WI, 53211.

Microbial symbioses are ubiquitous but the mechanisms underlying symbiont host range, transmission between generations, and contributions to host health or disease are not well understood. The mutualistic association between insect parasitic *Steinernema* nematodes and *Xenorhabdus* bacteria has emerged as a model to address such questions. The *Xenorhabdus-Steinernema*-insect symbiosis is experimentally tractable and versatile; molecular techniques and genomic sequences are available for *Xenorhabdus* bacteria, the nematode and insect hosts are inexpensive to rear, and *Xenorhabdus* mutualistic and pathogenic traits can be studied independently. Since their discovery studies have revealed that *Steinernema* depend on *Xenorhabdus* to overcome insect defenses and convert insect cadavers into nutrients necessary for development. *Xenorhabdus* bacteria also protect the insect cadaver from scavengers and microbes, including other *Xenorhabdus* species, through the production of secondary metabolites and bacteriocins. Expression of at least some of these activities is controlled by a phenotypic switch that is a conserved trait among *Xenorhabdus* spp. In *X. nematophila* the transcription factor Lrp controls this phenotypic switch and is a key regulator of transitions between mutualistic and pathogenic behaviors. Motility, exoenzyme and antibiotic production are coordinately controlled by the response regulator OmpR and the FliA and FliZ flagella regulatory proteins. These functions are expressed in the insect cadaver under iron-replete conditions and not in the iron-deficient nematode receptacle. The cost to the nematode for symbiont services is incurred in the infective juvenile (IJ) stage that carries bacteria as it migrates between insect hosts. Several studies have revealed that uncolonized IJs live longer than colonized IJs, indicating bacterial carriage is costly. In many cases, *Steinernema* nematode association with, and dependence on *Xenorhabdus* bacteria is strain or species specific. *X. nematophila* genes necessary for specific colonization of *S. carpocapsae* nematodes have been identified, and these were the first

host-range specificity determinants discovered in an animal-bacterium mutualism. Unlike the well-studied specificity mechanisms of rhizobium-legume symbiosis, the *X. nematophila* specificity determinants are derived traits, unique to *X. nematophila* among *Xenorhabdus*, raising the question of what molecular determinants dictate specificity in other *Steinernema-Xenorhabdus* associations. Progress has been made toward understanding how *Xenorhabdus* bacteria are transmitted between generations. The bacterial population that colonizes the luminal space between two anterior intestinal cells of IJs is essentially clonal. This finding is similar to recent observations in the *Vibrio*-squid symbiosis and supports theoretical models that predict host selective pressure to reduce competition among symbionts. Future research will likely be focused on implementing new technologies to study nematode and insect host responses to *Xenorhabdus*, and in expanding molecular genetic analyses to include a broader set of *Steinernema-Xenorhabdus* associations.

ENTOMOPATHOGENIC NEMATOLOGY SINCE THE 1990'S: THE OPENINGS OF A NEW ERA. **Parwinder S. Grewal.** Department of Entomology, The Ohio State University, Wooster, OH 44691, USA.

The early efforts (1923–1990) culminated in the establishment of entomopathogenic nematodes (EPNs) as legitimate biological pest control agents when the first commercial-scale liquid culture of *Steinernema carpocapsae* became fully operational in 1990. This created a great deal of excitement in the scientific community and the industry about the exceptional promise of EPNs for pest control. While the discovery of new target insects via the demonstration of EPN field efficacy continued, focus clearly shifted from the more descriptive to comparative studies and hypothesis driven research from the 1990s. Investigators began to include more than one nematode species in their studies and efforts to genetically improve the nematodes were initiated. Much progress has been made during the past 20 years. In this presentation, 20 seminal contributions by researchers in academia and industry which contributed to the establishment of EPNs and their symbiotic bacteria as model systems in biological control and biological sciences will be described. These will include the discoveries in EPN host-finding behavior, thermal adaptation, genetic selection for improved host finding, temperature tolerance and infectivity, construction of morphological and desiccation mutants, genetic transformation, molecular mechanisms of stress tolerance, pathogenicity, and symbiosis, development of water dispersible granules, and the establishment of the liquid culture of heterorhabditids, sequences of the genomes of the nematode and bacteria, bacterial toxins, and others.

BEHAVIOURAL ECOLOGY OF ENTOMOPATHOGENIC NEMATODES: PAST, PRESENT AND FUTURE. **Griffin, Christine T.** Department of Biology, National University of Ireland Maynooth, County Kildare, Ireland.

Behavioural studies of entomopathogenic nematodes (EPN; *Steinernema* spp. and *Heterorhabditis* spp.) have focussed more on the juveniles than the adults- in contrast to classical behavioural ecology. Information on dispersal and host-finding by infective juveniles (IJs) has contributed to the success of these nematodes as biological control agents. IJs' responses to host insects (whether live, dead or infected), and to host habitats (including damaged or undamaged plants), are crucial to the nematodes' own success as well as for the human user. Established paradigms on IJ behaviour include the concept of interspecific differences in foraging strategy, from ambush to cruise. Temporally plastic differences in infection behaviour between individuals of a given population have also been described. Although IJs are studied in groups, their social behaviour is not well known, but there are indications that they interact outside the host, whether in cooperation or competition. The effect of the bacterial symbiont (*Xenorhabdus* or *Photorhabdus*) on EPN behaviour cannot be ignored, and indeed the behaviour of the nematode can be considered a shared phenotype affected by nematode and bacterial genes. Some effects are obvious: bacterial products signal recovery from the dauer-like IJ state, affect the attractiveness or otherwise of the cadaver to late arriving nematodes (as well as to insect and avian scavengers), and influence dispersal of IJs from a spent host. Are there perhaps more subtle effects – for example, do the bacteria in an IJ's gut affect its behaviour? Since EPN reproduce within insect cadavers, it is the IJ that decides where reproduction is to take place, and its choice of host to invade also affects the pool of mating partners available to it when mature. The mating behaviour of *Steinernema* and *Heterorhabditis* has been described, but there is little further information on their sexual behaviour and its fitness consequences, such as choice or number of mating partners. There are some intriguing insights into the sex life of these nematodes, suggesting further avenues for study. For example, lone male steinernematids may survive a long time in hosts, but do not mature sexually unless a female arrives. It is assumed that this is adaptive. Behavioural traits are selected for their contribution to fitness, which is usually defined as lifetime reproductive success. For EPN, fitness is typically based on average numbers of descendents (after several generations) of groups of invading nematodes, rather than the progeny of individuals. Nevertheless, several studies have shown that the shaping of IJ behaviour has involved trade-offs with other life history traits. With their short life cycle and ease of culture, EPN are ideal model organisms, and the study of their behaviour increasingly addesses fundamental questions that do not directly impinge on biocontrol success.

LONG-TERM EFFECTS OF P FERTILISER ON NEMATODE COMMUNITY STRUCTURE ASSESSED BY MOLECULAR AND MORPHOLOGICAL METHODS **Griffiths[1], Bryan S., X.Chen[1], T.J.Daniell[2], R.Neilson[2] and V.O'Flaherty[3].** [1]Teagasc, Environment Research Centre, Johnstown Castle, Wexford, [2]James Hutton Institute, Dundee DD2 5DA, UK and [3]Dept Microbiology, National University of Ireland, Galway.

Grasslands are the main agricultural land use in Ireland, and are extensively fertilised to increase productivity. Phosphorus application in particular is of concern in relation to run-off losses and availability of P fertiliser. Effects of P availability on above-ground parameters such as vegetation composition and yield, and cattle performance, are well documented but effects on soil biology are less well known. Soil samples were taken from a long-term trial to study P for beef production started in 1968, with changes in 1999. Initially, three P treatments were set up, with fertilization rate of 0, 15 and 30 kg P $ha^{-1}$ $yr^{-1}$. In 1999, all plots were split to give an additional 3 treatments; 0-30; 15-5 and 30-0 where the first number is P applied from 1968-1999 and the second is P applied from 1999-2009. Nematodes were collected from soils sampled in Sept 2010 and identified with traditional morphology and with directed Terminal Restriction Fragment Length Polymorphism (T-RFLP) of extracted DNA. Total nematode density ranged from 15.4 to 23.4 $g^{-1}$ soil. Apart from the greatest P treatment (P30) there were no significant differences in nematode abundance. There were significant differences in the community structure of the nematodes. The most ecologically relevant of which was the shift from bacterial-feeding to fungal-feeding nematodes in the low-P treatments. While there were differences in the nematode community structure between the morphological and molecular methods, for example, fungi-feeding and predatory nematodes were underrepresented by T-RFLP both methods showed that the addition of P favoured bacterial-feeding nematodes, and increased Nematode Channel Ratio (NCR) that indicated a shift from fungal to bacterially dominated decomposition. Soil biology responds rapidly to the addition of P, but there is a lag-period before soils with a history of P fertilization show signs of P deficiency. PCR-TRFLP is well suited to monitoring nematode communities.

MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION OF *LONGIDORUS HANGZHOUENSIS* OCCURING IN CHINA. **Guo K., H. Shi and J. Zheng*.** Institute of Biotechnology, College of Agriculture and Biotechnology, Zhejiang University, Hangzhou 310058, P.R.China.

Needle nematodes (*Longidorus* spp.) are a group of ectoparasitic nematodes that not only affect root growth but also lead to root stagnation of an extensive range of crops by their feeding on plant root cells. Their great importance is on their ability to act as virus vectors and transmit viruses to many economically important crops. Comparing to other group of plant parasitic nematodes, fewer molecular data, which are useful for molecular identification, of longdorid nematodes are available in China. In this study, three *longidorus* populations, two were collected from the rhizosphere of *Metasequoia glyptostroboides* and *Catalpa ovata*, in Ningbo, Zhejiang Province, China and one was collected from the rhizosphere of *Pinus* sp.,in Hangzhou, Zhejiang Province, China. The morphology and morphometrics of females and juveniles were observed and measured, respectively. The results showed that the key morphological characters of the three populations are identical to *Longidorus hangzhouensis*, Zheng, 2001. Molecular analyses of three populations were carried out using rDNA region (ITS1, ITS2, near the full length of 18S gene and D2/D3 expansion segments of the 28S gene), and sequence length were 945 bp, 528 bp, 1778 bp and 886 bp, respectively. Phylogenetic analyses of *L. hangzhouensis* rDNA gene sequences with other *Longidorus* sequences published in GenBank were done using Bayesian inference. The phylogenetic relationships of these four independently evolving molecular markers, ITS1, ITS2, near the full length of 18S gene, and D2/D3 expansion segments of the 28S gene, supported *L. hangzhouensis* as a unique and valid species. This is the first report about the molecular data of *L. hangzhouensis*.

THE EFFECT OF RATE, TIMING AND APPLICATION METHOD OF VARIOUS INSECTICIDES FOR THE MANAGEMENT OF *HETERODER SCHACTII*. **Saad. L. Hafez[1], K. Luff[2].** [1]University of Idaho, [2]Bayer CropScience.

A field experiment was conducted to study the efficacy of various insecticides for the control of sugar beet cyst nematode in sugar beet. Temik 15G, Movento, Poncho Beta and Votivo were applied at different rates, alone or in various combinations. The experiment was established as a randomized complete block design with twelve treatments, each replicated five times. Temik was banded over the row at planting and again sequentially as a split application 8 weeks later. Seed treatments with Poncho Beta and Votivo were sown into Temik treated and non-treated plots. Foliar applications of Movento and Admire were made at 8 and 10 weeks after planting using a $CO_2$ powered plot sprayer. Nozzles were adjusted to deliver a 7 inch band over the row. Complete plant coverage with minimal runoff was achieved. Total yield of sugar beet roots was determined at harvest. Sugar beet yield in treated plots was higher compared to untreated plots. Foliar applications of Movento alone resulted in sugar beet yield similar to the Temik standard. Movento plus Admire Pro and both insecticides combined with Temik produced higher yield compared to Poncho Beta alone. Similarly, treatments with Poncho Beta combined with Votivo at

10 miu or Temik and both seed treatments combined with Temik resulted in greater yield as compared to Poncho Beta alone.

EFFICACY AND OPTIMAL TIMING OF MOVENTO FOR CONTROL OF COLUMBIA ROOT-KNOT NEMATODE ON POTATO, **Saad L. Hafez[1], K. Luff[2].** [1]University of Idaho, [2]Bayer CropScience.

A field experiment was conducted to determine the efficacy and optimal timing of Movento for the control of Columbia root-knot nematode in potatoes. Sequential combinations of Movento applied after Temik 15G or Mocap 6EC plus Admire Pro were also evaluated. All treatments were compared to a Telone II standard. The experiment was established as a randomized complete block design with eight treatments each replicated five times. Nematode densities averaged 500/500cc soil across the plot area. Telone was applied with a commercial applicator that ripped soil to a depth of 16-18 inch. Mocap was surface broadcast with a handheld $CO_2$ sprayer and double disc incorporated to a depth of 4 to 6 inches prior to planting. An Admire Pro at planting application (in furrow 4 inch band over the seed piece) followed the Mocap application. Temik was applied in furrow at planting. Movento foliar treatments were sprayed at various timings after emergence using a handheld $CO_2$ sprayer. All Movento treatments were tank mixed with Dyne-Amic @ 0.25% v/v. Potato cv. Ranger Russet seed pieces were planted, and at maturity tubers were hand-harvested and graded to determine total and infected yield. Data demonstrates that Telone, Movento (applied 14 and 28, 28 and 42 or 56 and 70 days after emergence) and the combination treatment of pre-plant Mocap followed by Admire Pro at planting and Movento (28 and 42 day after emergence) increased yield as compared to the untreated control. Nematode infected tubers in treated plots ranged from 1.7 to 39 percent. The lowest percentage of infected tubers occurred in plots treated with Telone (1.7%) or Mocap + Admire Pro + Movento (15%). Movento alone applied early in the growing season (14 and 28 or 28 and 42 days after emergence) resulted in less tuber infection than later applications (42 and 56 or 56 and 70 days after emergence). In general, combination treatments performed better than Movento alone in controlling tuber infection by *M. chitwoodi*.

PRODUCTION TECHNOLOGY AND FIELD APPLICATION OF ENTOMOPATHOGENIC NEMATODES IN CHINA. **Han, Richou.** Guangdong Entomological Institute, Guangzhou 510260, CHINA.

Entomopathogenic *Steinernema* and *Heterorhabditis* nematodes (EPNs) are the biological control agents which have the specific capacity to actively search and infect insect larvae in the soil. Recent advance in the solid and liquid production technology of EPNs has greatly improved the yield, quality, and shelf life of these beneficial worms in China, and stimulated the field application of these nematodes for the control of different insect pests. The factors influencing the production efficiency were explored, including medium development, optimization of the culture parameters (gas supply, inoculum size, bacterial status, temperature, etc), recovery of the infective juvenile (IJ) inocula, formation of the IJs, extraction and harvest of IJs. A company called Century Horse Development Ltd, under the guidance of Guangdong Entomological Institute, is running. Healthy and reasonable products in solid culture system are provided for field trials in China and for internal and international markets. Production cost is still a limiting factor for further commercial development in the world nematode-based market. Ideal pest targets for these nematodes were evaluated. Apart from the important insect pests (such as flee beetle *Phyllotreta striolata*, and chive midge *Bradysia odoriphaga*) in vegetables, new invasive pests such as the oriental fruit fly *Bactrocera dorsalis* (Hendel), asiatic palm weevil *Rhabdoscelus lineaticollis* (Heller) and banana moth *Opogona saccchari* (Bojer) were also controlled by EPNs.

EFFECT OF AQUEOUS EXTRACT OF SIX PLANT MATERIALS ON EGG HATCH AND MORTALITY OF *HETERODERA GLYCINES*. **Hassan, M.A. and J. Zheng.** Institute of Biotechnology, College of Agriculture & Biotechnology, Zhejiang University, Hangzhou 310058, P.R.China.

Experiments were conducted in the laboratory and greenhouse to determine the effect of aqueous extract of *Acasia nilotica, Azadirachta indica, Brassica spp, Ecklonia maxima Saccharum officinarum* (molasses) *and Sesamum indica* on the egg hatch and J2 mortality of *Heterodera glycine*. In vitro laboratory experiments, cold water extract of the leaves and/or seeds (kernel) of the botanicals hindered the hatch of *H. glycines* eggs after 24 hours and 48 hours exposure. Exposure of J2 for 24 hours and 48 hours resulted in 57 to 65% mortality and 75 – 85% mortality respectively. Green-house, *in vivo* bioassay pot experiment was conducted using soyabean (*Glycine max*) inoculated with 2000 J2 larvae. Grounded fine powder of the six botanicals were tested at four concentrations 1%, 2%, 5% and 10% w/w to sterilized (autoclaved) soil .The botanical exhibited between 48 – 85% J2 mortality when compared to non-amended pods where no botanical was added. The efficacy of the botanical control of nematodes is remarkable. This could give rise to sustainable management of plant parasitic nematodes that is safe, cheap and environmentally-friendly.

*IN VIVO* QUANTIFICATION OF MITOCHONDRIAL FORM AND FUNCTION IN *CAENORHABDITIS BRIGGSAE* NEMATODES HARBORING A NATURALLY OCCURRING mtDNA DELETION. **Hicks[1], Kiley A., D.R. Denver[2], D. K. Howe[2], A. Leung[2], and S. Estes[1].** [1]Biology Department, 1719 SW 10th Ave, Portland State University, Portland, OR 97201, [2]Department of Zoology, 3029 Cordley Hall, Oregon State University, Corvallis, OR 97331.

Mutations affecting the mitochondrial respiratory chain (MRC) have been linked to organismal aging and several human pathologies. Radical alterations of mitochondrial physiology and morphology often accompany such mutations. However, the complex relationships between MRC dysfunction and mitochondrial physiology and morphology remain enigmatic. Natural populations of *Caenorhabditis briggsae* nematodes harbor a large, heteroplasmic mitochondrial (mtDNA) deletion within the *ND5* gene, the normal product of which is an integral subunit of MRC Complex I. The deletion is expected to reduce MRC efficiency in animals containing high *ND5* deletion percentages. We performed the first *in vivo* assessment of natural variation in mitochondrial ROS levels, membrane potential, and several aspects of organelle morphology using mitochondria-specific fluorogenic dyes and confocal microscopy. We conducted the same analyses on mitochondrial-nuclear hybrid strains to test the effects of mtDNA vs. nuclear variation on the measured mitochondrial shape and functionality traits. We found significant natural variation in all mitochondrial physiology and morphology traits, and contrary to our expectations, that this variation was non-linearly associated with *ND5* deletion heteroplasmy level. Specifically, ROS levels and membrane potential tended to be lower in animals with intermediate frequencies of deletion bearing mtDNA genomes, but increased dramatically when deletion level exceeded 50%. We also identified aspects of mitochondrial morphology that are associated with high *ND5* deletion levels. Finally, hybrid strain analyses provide strong support for the idea that the natural among-population variation in the measured phenotypes owes itself to mtDNA, not nuclear genetic, variation. Together, our findings suggest that mitochondrial threshold effects may contribute to among-population phenotypic variation.

DECREASE IN BIOMASS OF ENERGY CROPS DUE TO PLANT PARASITIC NEMATODES. **Hillnhütter, Christian[1], T. Mekete[3], K. Reynolds[1], M. Gray[1,2] and T. Niblack[1,2].** [1]Energy Biosciences Institute, University of Illinois, 1206 W. Gregory Dr., Urbana, IL, 61801, [2]Department of Crop Sciences, University of Illinois, 1102 S. Goodwin Ave., Urbana, IL 61801, [3]Entomology and Nematology Department, University of Florida, 970 Natural Area Dr., Gainesville, FL 32611.

Two field experiments were established to estimate the impact of plant parasitic nematodes on biomass yield of *Miscanthus* × *giganteus* (MXG) and switchgrass. The field plots were situated near Havana (IL) on a sandy loam soil. Prior to planting half of the experiment was treated with Telone II, a fumigant nematicide, to reduce the nematode population; the other half was left untreated as a control. Two weeks later the plants were transplanted from greenhouse pots to the field. Nematode soil samples were collected one week after planting and at the end of the growing season from each plant. Nematodes were extracted from the soil, counted and identified to the genus level. During October the plants were measured for height and then harvested in order to determine fresh and dry biomass yield. Nematodes of the genera *Criconemella*, *Helicotylenchus*, *Heterodera*, *Hoplolaimus*, *Longidorus*, *Pratylenchus*, *Tylenchus,* and *Xiphinema* were found in the soil samples. The nematicide treatment significantly reduced the number of nematodes relative to the control for most of the genera. The height of the control MXG was significantly reduced compared to nematicide treated rows. Furthermore, the biomass yield of treated switchgrass was significantly higher than untreated switchgrass. Also, a significant negative correlation was found between the *Pratylenchus* population and biomass of switchgrass. Results indicate a significant negative effect of plant parasitic nematodes on the biomass accumulation of the energy crops MXG and switchgrass. The experiment is designed to be long term. These results represent the first year of data. In the coming years, the influence of the two crops on nematode population development will be investigated, as will the effect of nematode dynamics on the perennial growth of MXG and switchgrass.

VISUALIZATION OF BELOWGROUND DAMAGE CAUSED BY *HETERODERA SCHACHTII* AND *RHIZOCTONIA SOLANI* ON SUGAR BEET BY NUCLEAR MAGNETIC RESONANCE IMAGING. **Hillnhütter[1], Christian, R.A. Sikora[1], E.-C. Oerke[1] and D. van Dusschoten[2].** [1]Institute of Crop Science and Resource Conservation (INRES) – Phytomedicine, Rheinische Friedrich-Wilhelms-Universität Bonn, Nussallee 9, 53115 Bonn, Germany, [2]ICG-3: Phytosphere, Forschungszentrum Jülich, 52425 Jülich, Germany.

Belowground symptoms caused by *Heterodera schachtii* often include the development of compensatory secondary roots and beet deformity that can be seen following destructive removal of entire root systems from soil. Brown to black decay on the beet and roots are symptomatic when plants are infected with *Rhizoctonia* crown and root rot, caused by *Rhizoctonia solani*. Nuclear magnetic resonance imaging was applied for detection of belowground symptoms caused by *H. schachtii* and/or *R. solani* on sugar beet. Excessive lateral root development and

beet deformation of the nematode treated plants was obvious on magnetic resonance images. Three dimensional resonance images gave in sight on cysts of *H. schachtii* attached to the roots in the soil. *Rhizoctonia* crown and root rot on the beet was detected by lower signal intensity (water content) in resonance images at sites where rotting occurred. The disease complex of both organisms together resulted in *Rhizoctonia* crown and root rot development on the site of nematode penetration. Synergistic damage was observed due to interaction of both pathogens. Magnetic resonance of plants can give new insights into pathogenesis of plant damaging organisms and will be valuable for breeding tests.

EVOLUTION AND PHYLOGENETICS OF *CAENORHABDITIS* MITOCHONDRIAL GENOMES. **Howe, Dana K., L.J. Wilhelm, J. Campbell, K. Clark, and D.R. Denver.** Dept. of Zoology and Center for Genomic Research and Biocomputing, Oregon State University, Corvallis, OR 97331.

Numerous new nematode species in the genus *Caenorhabditis* have recently been discovered as a consequence of increased global sampling efforts. To characterize the relationships between species in this genus, we sequenced the mitochondrial genomes from 45 *Caenorhabditis* nematodes, at least one from each of 24 species. To accomplish this, mitochondrial DNA was PCR amplified in two large, overlapping amplicons, labeled with unique barcoded adapters, and sequenced in parallel on one lane of an Illumina high-throughput sequencing system. The individual sequences reads were assembled into complete or nearly complete mitochondrial genomes using SCRAPE, a Perl application that combines multiple assembly programs. The 36 nematode mitochondrial DNA genes (12 protein coding, 22 transfer RNAs and two ribosomal RNAs) were identified using homology based methods, annotated and used for downstream phylogenetic analyses. We compared our mitochondrial DNA phylogenies to published nuclear phylogenetic analyses of this nematode genus. Four *Caenorhabditis* species were discovered to encode large non-coding mitochondrial DNA elements. Many of these elements were observed to accumulate in a common cluster of four tRNA genes. Our study provides a novel mito-genomic approach to investigating evolutionary processes that is amenable to extension to other nematode taxa.

ENTOMOPATHOGENIC NEMATODE ECOLOGICAL MODELING, FROM FRONTIERS OF ECOLOGY TO THE FUTURE OF AGRICULTURE. **Casey W. Hoy and Parwinder S. Grewal.** The Ohio State University, Ohio Agricultural Research and Development Center.

Contributions to both fundamental and applied questions in ecology have accrued from research focused on the complex population dynamics and interactions among entomopathgenic nematodes (EPNs), their insect host communities and the soil environment. Among the qualities of EPNs that make their complex roles in ecosystems particularly challenging to investigate, but rewarding in understanding gained, are: 1. unique population dynamics; 2. complex environmental interactions in a range of habitats; 3. roles and relationships in complex but cryptic soil food webs; 4. potentially rich, diverse, and currently poorly described host communities; 5. complex spatial dynamics occurring at nested scales of aggregation and dispersal; 6. apparent metapopulation dynamics with complex tradeoffs in reproduction, survival, and dispersal. Contributions to a number of the key frontiers in ecology have come from quantitative research on EPN ecology, and further contributions will become increasingly important for sustainability and resilience of global agriculture. Quantitative ecologists studying EPNs have made contributions to the frontiers in ecology. Insight into the dynamics of coalescence in complex communities has arisen from a number of recent investigations on the complex sets of habitat conditions that jointly affect EPNs, plant communities, and their associated insect communities and food webs, using multivariate statistical modeling. As the evidence regarding their ecological roles in these communities accumulates, so too do hypotheses regarding the evolutionary and historical determinants of the ecological processes in which EPNs function. Simulation models based on EPN biology reveal emergent properties from their complex dynamics. When the focus shifts from nematode populations to communities, food webs and habitats, the complexity as well as pattern emergence from that complexity, increases as well. We have studied EPNs at a wide range of spatial and temporal scales, despite the fact that direct observation of these microorganisms is possible only at very small scales. Novel advancement of understanding of ecological topologies is possible through the ongoing challenge of connecting the relatively small scales of individual nematode activity with the potentially much larger scales of activity of their insect hosts, and finally with the more continental and global scales of their soil, plant and insect communities and their geophysical interactions. As we look forward to future contributions from the quantitative study of EPNs, none are more important than addressing the challenges facing global agriculture. Our current research is focused on how these native soil organisms can be exploited to maximize food productivity and minimize environmental impacts of pest control. EPN population modeling coupled with empirical tests of specific strategies can provide needed answers to the benefits that sustainable soil management can deliver for both agricultural production and other ecosystem services. Resilience of agricultural systems must be improved to deal with both gradual climate change and increased climatic variability and extremes. EPNs are ubiquitous across a range of

habitats and appear to be well adapted already to local extinction and recolonization, and may therefore provide a unique example of a soil microorganism that improves agroecosystem resilience despite extreme changes.

OCCURRENCE AND DAMAGE POTENTIAL OF PLANT-PARASITIC NEMATODES ASSOCIATED WITH BLUEBERRIES (VACCINIUM SPP.) IN GEORGIA AND NORTH CAROLINA. **Jagdale,[1] Ganpati B., T. Holladay[1], P. M. Brannen[1], B. Cline[2], A. P. Nyczepir[3] and J. P. Noe[1].** [1]Dept. of Plant Pathology, University of Georgia, Athens, GA, 30602, [2]Dept. of Plant Pathology, North Carolina State University, Horticultural Research Station, Castle Hayne, NC 28429 and [3]USDA-ARS Southeastern Fruit & Tree Nut Res. Lab. 21 Dunbar Road, Byron, GA 31008.

Blueberry replant disease is an emerging threat to continued blueberry (*Vaccinium* spp.) production in Georgia (GA), and possibly in the southeastern U.S. including North Carolina (NC). Blueberry replant disease symptoms appear to be similar to those seen in peach tree short-life (PTSL) disease, which is known to be caused by the ring nematode (*Mesocriconema xenoplax*). After determining that ring nematodes are pathogenic to blueberry in GA, we conducted a more extensive and systematic survey of plant-parasitic nematodes (PPNs) infesting commercial blueberry farms and to determine the severity and extent of nematode replant disease in blueberry in GA and NC during the 2010 growing season. We found that six PPN genera including *Mesocriconema* spp., *Tylenchorhynchus* spp., *Hoplolaimus* spp., *Hemicycliophora* spp., *Dolichodorus* spp. and *Xiphinema* spp. were associated with blueberry in both GA and NC. *Paratrichodorus* spp. was found only in GA. The most frequently detected PPN in GA, *Mesocriconema* spp. was found in nearly half of the blueberry farms with a mean population density of 290/100 $cm^3$ soil in those samples that contained *Mesocriconema* spp. In NC, *Dolichodorus* spp was the most frequently encountered nematode. We also found that in GA the overall mean population density recorded for ring nematodes was higher than population levels that were previously observed in the replant disease areas in the field. Our results suggest that the blueberry replant disease could become a major limitation to continued production on existing GA farms. In NC, we found that the *Dolichodorus* spp. was predominantly associated with blueberry but its pathogenicity to blueberry and relationship with blueberry replant disease are unknown, and warrant further investigation.

MOLECULAR MARKERS FOR IDENTIFICATION OF *MELOIDOGYNE* INFECTING GUAVA IN THAILAND. **Jindapunnapat, Kansiree[1], B. Chinasri[1], and B. Sipes[2].** [1]Department Plant Pathology, Kasetsart University, Bangkok, Thailand, and [2]Department of Plant and Environment Protection Sciences, University of Hawaii, Honolulu, USA.

The root-knot nematode *Meloidogyne incognita* is an important plant-parasitic nematode and was first reported in guava in Thailand about 20 years ago. The nematode causes substantial yield loss. Development of a molecular marker specific to root-knot nematode in guava will allow for rapid and correct identification and detection before seedlings are distributed from nurseries. *Meloidogyne* was compared with *Pratylenchus, Rotylenchulus, Helicotylenchus, Aphelenchus* and *Hopolaimus* collected in guava fields and entomo-pathogenic nematode *Steinernema*. The 16s ribosomal RNA (16s rRNA) gene and Cytochrome Oxidase II (COII) gene were used to develop molecular markers. These genes were aligned and characterized for intraspecific genetic variation in *Meloidogyne*. UNI_16sM and UNI_COIIM were developed with lengths of 400 bp and 150 bp respectively. The primer UNI_16sM and UNI_COIIM were designed to detect specific *Meloidogyne* from a portion of the conserve region in the mitochondria. The primer Nema_18s was designed to be specific to Phylum Nematoda and amplify a 1 kb fragment. Primer Nema_18s was compared with Primer UNI_16sM and UNI_COIIM, and had similar results in detecting *Meloidogyne*. Nema_18s can amplify the other genera whereas UNI_16sM and UNI_COIIM cannot amplify those genera. Therefore UNI_16sM and UNI_COIIM are specific to *Meloidogyne* and do not detect other genera found in guava in Thailand. UNI_16sM and UNI_COIIM are able to prime DNA that is damaged, incomplete low concentrations. UNI_16sM and UNI_COIIM are specific to and efficient with *Meloidogyne* compared with other genera found in guava in Thailand.

A NEW PARASITISM GENE Mj-D15 ISOLATED FROM *MELOIDOGYNE JAVANICA*. **Jinling, Liao[1], B. R. Lin[1] and K. Zhao[1].** [1]College of Natural Resource and Environment, South China Agricultural University, Guangzhou, PR China, 510642. Contact person: jlliao@scau.edu.cn

Meloidogyne javanica is one of important pathogens of crops, for infecting many kinds of tropical crops and causing a great losses. A new gene Mj-D15 isolated from the esophageal gland cells of M. javanica was herein reported. The full-length cDNA and DNA were obtained. ORF is 822 bp coding 274 aa, while DNA contains two introns and three extrons. RT-PCR analysis from nematode developments suggested Mj-D15 was not expressed in adult females but in the periods of dual~multi cells, blastula, protogaster, J1 and J2 stages. The immunolocalization indicated that it expressed in the esophageal gland cells. The experiment of dsRNA soaking was conducted and it showed the infection rate of the second stage juveniles to the tomato was significantly decreased. The expression construct of pTRV was obtained and transformed to the tobacco. The infection of the second stage juveniles, gall index, egg masses and the size of adult females were all decreased significantly after 6 d, 12 d, 21 d and

45 d of inoculation with nematodes. It can be concluded from present work that Mj-D15 is related to the parasitism and infection of M. javanica.

RNAI SILENCING OF THE *MELOIDOGYNE INCOGNITA RPN7* GENE REDUCES NEMATODE PARASITIC SUCCESS. **Niu Junhai [1,2], H. Jian[1]*, J.M. Xu[1], C.L. Chen[1], Q.X. Guo[1], X.Y. Wang[1], Q. Liu[1], Y.D. Guo[2].** [1]Key Laboratory of Plant Pathology (MOA); Department of Plant Pathology, China Agricultural University, Beijing 100193, P. R. China; [2]Department of Vegetable Science, China Agricultural University, Beijing 100193, P. R. China.

RNA interference (RNAi) provides a powerful tool for analyzing gene function in plant-parasitic nematodes, offering the possibility of identifying new nematode targets for developing novel forms of nematode resistance in crop plants. To validate a potential of targeting *Mi-Rpn7*, a gene homologue involved in proteasome regulation in *C. elegans*, for generating resistance against the root-knot nematode *Meloidogyne incognita*, and to evaluate our modified protocols for assessing silencing phenotypes, we used *in vitro* and *in vivo* RNAi approaches to silence the *Mi-Rpn7* gene in *M. incognita*. Soaking pre-parasitic second-stage juveniles (J2) in solution containing truncated *Mi-Rpn7* dsRNA (408 bp) led to a specific reduction of *Mi-Rpn7* transcripts. The treated J2s showed an interrupted locomotion revealed by an attraction assay on the Pluronic gel medium. Inoculation of tomato roots with treated J2s resulted in a reduction of nematode infection rate ranging from 55.2–66.5% when compared with inoculations with control-treated nematodes. To evaluate if *Mi-Rpn7* has a role in nematode development and parasitism, composite soybean plants with *Mi-Rpn7* dsRNA expression in the roots were generated and tested for nematode infection. Four weeks after nematode inoculation, the composite plants showed an approximately 50.8% ($P < 0.05$) reduction in the number of nematode eggs per gram root when compared with the infected control plants. These results demonstrated that *Mi-Rpn7* has a role in nematode motility and infectivity, and that *in planta* delivery of RNAi of *Mi-Rpn7* reduces plant susceptibility to *M. incognita* infection.

A SURVEY OF ROOT LESION NEMATODE (*PRATYLENCHUS* SPP.) IN THE DRYLAND WHEAT PRODUCTION AREAS OF EASTERN WASHINGTON. **Kandel[1], Shyam L., A.A. Elling[1], R.W. Smiley[2], and T.C. Paulitz[1].** [1]Dept. of Plant Pathology, Washington State University, Pullman, WA 99164, [2]Oregon State University, Columbia Basin Agricultural Research Center, Pendleton, OR 97801.

Root lesion nematodes (*Pratylenchus neglectus* and *P. thornei*) are common in dryland wheat production areas of the Pacific Northwest (PNW) of US and can significantly reduce yield. Currently available wheat varieties in the PNW are susceptible to nematode damage. The objectives of the research were to determine how the root lesion nematodes are distributed throughout the wheat production region in the Washington and optimization of quantitative PCR reactions using species-specific primers for species identification and quantification in soil. A field survey was conducted by collecting 90 soil samples from Washington State University wheat variety trials and growers' fields during summer 2010 including (1) wheat-summer fallow area of Ritzville/Connell, (2) Garfield County-Walla Walla, (3) the Palouse from Uniontown to Spokane, and (4) the northwest growing area (Reardan-Wilbur, Waterville Plateau, Douglas County). Root zone soil from 20 to 25-cm depth was collected from three locations in each field. All collected samples were sent to Western Laboratory, Parma, ID to extract plant-parasitic nematodes. Nematodes were extracted, morphologically identified to the genus level, quantified in each sample, and collected in about 10-ml water suspension. Morphological counts varied from 0 to 5160 nematodes per kilogram of soil. Higher counts were found in the Palouse region from Uniontown to Spokane where a winter wheat-spring wheat/spring legume rotation is dominant. Each sample was centrifuged to concentrate nematodes in a small volume. DNA was extracted with different DNA extraction buffers and Mo Bio soil DNA isolation kit with some modification. Optimization of quantitative PCR was done using species-specific primers to determine nematode population in soil. Nematode genomic DNA extracted using Mo Bio Kit and KCl-lysis buffer was amplified for *P. thornei* using species-specific forward and reverse primers THO-ITS-F2/THO-ITS-R2 in conventional PCR. DNA extraction by Mo Bio Kit generated specific amplification for *P. thornei* in quantitative PCR. However, correlation between nematode morphological count and cycle number in quantitative PCR was not seen. Use of species-specific primers with PCR allows precise identification of species and improvement of quantitative PCR procedures become very useful as a management tool to assess risk and to phenotype germplasm when breeding for resistance.

SUSCEPTIBILITY OF RED POTATO CULTIVARS ( *SOLANUM TUBEROSUM L.*) TO THE ROOT-KNOT NEMATODE *MELOIDOGYNE INCOGNITA*. **Kandouh, Basil and B. Sipes.** Department of Plant and Environmental Protection Sciences. University of Hawaii at Manoa. Honolulu, 96822.

Yield loss in some potato cultivars (*Solanum tuberosum* L.) by *Meloidogyne* spp. (root knot nematodes) may reach 80% in tropical and subtropical areas. Susceptibility of red potato cultivars to *Meloidogyne incognita* is generally

unknown and requires investigation to appropriately manage yield losses. In a greenhouse, four population densities of *M. incognita* were evaluated on four red skinned potato cultivars. Tolerance and resistance to the nematode were evaluated ($P \leq 0.05$) by using tuber weight (TW) and nematode reproduction factor (Rf) (Rf = Pf/Pi) respectively. Cultivars Mountain Rose and Desiree had TW above the overall mean by 18% and 16%, respectively. TW was less than overall mean for Pink Pearl and Red Thumb (15% and 20%, respectively). A ranking of Rf showed that Desiree allowed the greatest nematode reproduction. Pink Pearl and Red Thumb were also consistently among the cultivars supporting a high Rf regardless of Pi ($Rf \geq 1$). Rf was suppressed in cultivar Mountain Rose ($Rf < 0.1$). In the susceptible-intolerant cultivars Red Thumb and Pink Pearl, TW was reduced even at low initial populations (Pi = 20 or 200 eggs/plant). Cultivar Desiree was susceptible-tolerant and the highest Pi (2000 eggs/plant) did not affect TW. Cultivar Mountain Rose was resistant-tolerant and not only suppressed Rf but also had an increase the TW with nematode infection. Resistance to *Meloidogyne incognita* is in some of red skinned potato cultivars. This information is for breeders and growers in selecting cultivars tolerant and resistant to *M. incognita*, mitigating yield loss.

"REVERSE TAXONOMY" FOR ELUCIDATING DIVERSITY OF TERMITE-ASSOCIATED NEMATODES. **Kanzaki, Natsumi[1,2], R. M. Giblin-Davis[1], R. H. Scheffrahn[1], H. Taki[2], A. Esquivel[3], K. A. Davies[4] & E. A. Herre[5].** [1]University of Florida/IFAS, Fort Lauderdale Research & Education Center, 3205 College avenue, Davie, FL 33314, [2]Forest Pathology Laboratory, Forestry & Forest Products Research Institute, 1 Matsunosato, Tsukuba, Ibaraki 305-8687 Japan, [3]Universidad Nacional, Escuela de Ciencias Agrarias, Laboratorio de Nematologia, Apto. 86-3000, Heredia, Costa Rica, [4]Centre for Evolutionary Biology and Biodiversity, School of Agriculture, Food and Wine, The University of Adelaide, Waite Campus, PMB 1, Glen Osmond, SA 5064, Australia, [5]Smithsonian Tropical Research Institute Box 0843-03092 Balboa, Ancon, Republic of Panama.

"Reverse taxonomy" is a method of evaluating the biodiversity of microorganisms that were first elucidated using MOTUs (Molecular Operational Taxonomic Unit) by tying back to targeted organisms (= unknown species) which were re-isolated from the microniche from which they were originally sequenced. The MOTUs allow construction of a species list including nominal and unknown species relative to matches in a reference sequence database. To test the practical applicability of "reverse taxonomy" for entomophilic nematodes, termite-associated nematodes were surveyed as a model system. Forty eight species (298 colonies) of termites were collected from the American tropics and subtropics and 20 individual workers from each of 259 colonies were dissected in a water drop to collect nematode MOTUs, and 20 additional workers were dissected onto water agar and kept at room temperature to attempt the establishment of nematode cultures for morphological identification. The MOTU survey yielded ca. 130 MOTUs which were separated into 41 sequence types (= species) belonging to 15 tentative genera from 159 individual nematodes. Culturing attempts revealed 20 MOTUs including 14 species (six genera) of pure-cultures, which were identified both morphologically and molecularly. Ten of the MOTUs overlapped between straight sequencing attempts and cultured isolates. Within 15 tentative MOTU genera, four were identified to the genus-level, which is useful as a functional diversity survey tool, solely by molecular sequence comparisons with the database, and an additional six were identified from the cultured materials, but the others were not identified. This is probably due to a lack of molecular data for the nematodes in the sequence database at the time (= 2008), and the situation has not changed drastically in 2011. "Reverse taxonomy" which entails traditional taxonomic methods combined with isolation, culturing attempts and observation appear to be indispensable for improving the MOTU approach by helping to significantly expand and accurately voucher the extant molecular database.

MULTIGENE PHYLOGENY OF NEMATODA. **Koutsovoulos[1], Georgios D., M.O. Jones[1], M.L. Blaxter[1].** [1]Institute of Evolutionary Biology, University of Edinburgh, Edinburgh, United Kingdom.

The reconstruction of phylogenetic relationships within the phylum Nematoda has been difficult because of morphological conservation and the high rate of homoplasy between species. Furthermore, adaptive radiations can include periods of rapid cladogenesis which require a large amount of molecular data to resolve. So far, phylogenetic trees based on molecular data have used only ribosomal and/or mitochondrial gene sequences. However, portions of the nematode tree remain unresolved, indicating the need to incorporate phylogenomic methods to improve topology resolution. For our analysis, we constructed a dataset covering many tens of genes using EST sequences from NEMBASE4, sequences from transcriptome analysis, and sequences from whole genome sequence projects. Due to the bias in sequencing towards nematodes from clades III-IV, the species used in our analysis do not yet span the diversity of the phylum in a balanced way. Even so, the phylogenetic tree, obtained by analyzing the supermatrix with maximum likelihood and Bayesian inference methods, is largely congruent with the existing molecular-based studies.

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RELIABILITY OF MELDOLA'S BLUE STAINING METHOD TO TEST *GLOBODERA* EGG VIABILITY. **Kroese[1], Duncan, I.A. Zasada[2], and R.E. Ingham[1].** [1]Department of Botany and Plant Pathology, Cordley 2082, Oregon State University, Corvallis, OR 97330, [2]USDA-ARS Horticultural Crops Research Laboratory, 3420 NW Orchard Ave., Corvallis, OR 97330.

Determination of egg viability is important when considering the potential threat following *Globodera* cyst detection in the field. Laboratory-based methods to test viability include staining with Meldola's Blue (MB) and/or juvenile (J2) hatching assays (HA) using potato root diffusate (PRD). Viability staining with MB is the preferred method for most laboratories because the production of PRD is not required. However, MB staining and HA approaches have not been tested under conditions to directly compare their assessments of *Globodera* egg viability. Using two bioassay strategies, cysts from an atypical *Globodera* population found in Oregon were subjected to both viability assessment methods. In strategy one, intact cysts were first exposed to MB stain for one week and the numbers of stained and unstained eggs were determined. Eggs were then transferred to PRD, and one week later the numbers of J2 and remaining eggs were determined. In the second strategy, intact cysts were exposed to PRD for one week, the numbers of J2 and remaining eggs were determined per cyst, then the eggs were transferred to MB stain. After one week, the numbers of stained and unstained eggs were determined. Two different cohorts of cysts were evaluated using these experimental strategies. Cohort 1 comprised cysts produced on potato in the greenhouse, exhibiting low J2 hatch when exposed to PRD. Cohort 2 were field-collected cysts which yielded significant J2 hatch when exposed to PRD. Percentage viability is expressed as the number of hatched J2 or unstained eggs/total number of eggs within a cyst. With field-produced cysts, MB staining and HA methods produced similar viability estimates, with averages of 67.9% and 71.3%, respectively. In contrast, greenhouse-produced cysts yielded much lower and unequal estimates. MB staining estimated egg viability at 35.8%, whereas the HA method estimated viability at 6.8%. In addition J2 hatch from unstained (viable) greenhouse eggs was 15.7% after PRD exposure compared to 64.7% for field eggs. Significance of the two strategy findings, and the possible role of diapause, are discussed.

ASSEMBLING AND ANNOTATING HALF A DOZEN DRAFT NEMATODE GENOMES USING INEXPENSIVE ILLUMINA SEQUENCING. **Kumar, Sujai[1], G. Koutsovoulos[1], and M. Blaxter[1].** [1]Institute of Evolutionary Biology, University of Edinburgh, EH9 3JT, UK.

Illumina sequencing currently provides the best cost to quality ratio of second generation sequencing technologies for de novo sequencing. High quality draft genomes and annotations for most genes can be created at extremely low cost (a few thousand dollars per genome) with very short turnaround times (as little as two months from worm to WormBase). We present several nematode genomes that we are currently working on: where they sit in the phylum, the kind of data we have for each species, costs in terms of money and staff time, the level of completeness of each genome, and the kinds of questions we can and cannot answer with these draft genomes. There are some disadvantages of using only short reads - they cannot resolve repeated regions longer than the insert length and the assembled contigs are typically more fragmented than first-generation sequencing projects. However, we demonstrate that additional RNA-seq and mate pair data improve the annotations and assemblies significantly. We will also discuss the bioinformatics pipelines used and the technical aspects of identifying and dealing with low quality sequencing data, contaminants, non-genomic reads, and sequencing artifacts.

STRAND-SPECIFIC NUCLEOTIDE COMPOSITIONAL BIAS (SKEW) IN NEMATODE MITOCHONDRIAL DNA REVEALS TAXONOMIC CLASS DISTINCTION. **Lewis[1], S.C., and B. C. Hyman[1,2].** [1]Graduate Program in Genetics, Genomics, and Bioinformatics and [2]Departments of Biology and Nematology, University of California, Riverside, CA 92521.

Recent comparative studies have revealed striking differences in mitochondrial genome architecture between the two major nematode taxonomic classes. While genome size, the use of only one mitochondrial DNA (mtDNA) strand to encode genes, and syntenic gene orders are conserved features among Chromadorean mitochondrial genomes, Enoplean mtDNAs are characterized by lengthy sequence duplications, inversions, the use of both mtDNA strands to encode genes, and a paucity of gene order conservation. To investigate how these structural differences may influence, or be influenced, by patterns of nucleotide composition, we compared AT-richness and strand-specific nucleotide compositional bias (skew) among all available complete nematode mtDNA sequences. While Chromadorean mtDNAs are consistently near 74% AT, the AT content of Enoplean mtDNAs varies from 74-82 %. Like most metazoans, Chromadorean mtDNAs exhibit strong nucleotide compositional skew between mtDNA strands. In contrast, negligible skew is observed in Enoplean mtDNA, regardless of the transcriptional polarity of resident genes on each strand. These patterns persist when skew analysis is restricted to third codon positions or fourfold-degenerate sites of mitochondrial protein coding sequences, nucleotide positions likely immune to purifying selection. Comparison of cumulative skew plots between Enoplean congeners with differing gene orders reveals definable inflection points that directly correspond to changes in transcriptional polarity of the mitochondrial genes. These findings suggest that the high-frequency of mitochondrial genome rearrangement characteristic of Enoplean mitochondrial genomes may counteract strand-specific accumulation of nucleotide substitutions that contribute to skew within Chromadorean mtDNAs. Alternatively, rearrangements may be tolerated more easily in mitochondrial genomes lacking compositional bias between DNA strands.

USING THREE FUNGIVOROUS NEMATODES TO CONTROL LETTUCE DAMPING-OFF DISEASE CAUSED BY RHIZOCTONIA SOLANI (AG4). **Li[1], Yen-Ting, P. Chen[1], and T. T. Tsay[1].** [1]Dept. of Plant Pathology, National Chung Hsing University, 250 Kuo-Kuang Rd. Taichung 402, Taiwan.

Fungivorous nematodes have been reported as potential biological control agents to suppress soil-born diseases. Three fungivorous nematodes; including Aphelenchus sp., Aphelenchoides composticola, and Parapehelenchus sp. were isolated from the rhizosphere of Luffa cylindrica, Acacia confusa, and grass, respectively, and tested in this study. Nematodes cultured under the temperature ranging from 16 °C to 36 °C were evaluated for the optimal temperature based on the reproduction rate. The best temperature for reproduction of Aphelenchus sp. was 32~36 °C, Aphelenchoides composticola was 24 °C, and Parapehelenchus sp. was 28 °C. To evaluate their potential as biocontrol agents and their host range, ten plant pathogenic fungi and three saprophytic fungi were used in the test. The population of Aphelenchus sp. increased 8 to 16 times when cultured on 5 phytopathogenic fungi and a Trichoderma strain, a commercialized biocontrol agent. All three nematodes had the largest population when cultured on Rhizoctonia solani (AG4). Therefore, their ability to control lettuce Rhizoctonia damping-off disease was tested in the greenhouse. Rhizoctonia solani was grown on 90 mm PDA plates for 4 days at 28 °C, and 1 plate was mixed with 4 kg sterilized soil as the artificially inoculated soil. The infested soil was incubated at room temperature for 2 weeks allowing pathogen population to increase. In a 3 inch pot, 250 g infested soil was mixed with different concentrations of the fungivorous nematodes and incubated for 1 week at 28 °C before the seedlings were planted. Plants in the pots which were treated with 2,500 Aphelenchus sp. or Parapehelenchus sp. had 80%~88% survival rates, and those treated with 250 Aphelenchoides composticola had 92% survival rate. These results were significantly better than the 4% survival rate in the treatment without fungivorous nematodes. Aphelenchus sp., Aphelenchoides composticola, and Parapehelenchus sp. in this study had the potential to develop into biocontrol agents.

DAMAGE POTENTIAL OF *PRATYLENCHUS PENETRANS* TO SOYBEAN. **MacGuidwin, A. E.** Plant Pathology Dept 1630 Linden Drive, University of Wisconsin, Madison, WI 53706.

*Pratylenchus* spp. are common in agricultural fields in Wisconsin with incidence as high as 99% in a survey of 154 corn fields in 2007. The risk potential of *Pratylenchus spp.* for soybean is not known because most samples from soybean fields are assayed for cyst nematodes using methods inappropriate for *Pratylenchus spp.*. We conducted studies in 2009 and 2010 to test the hypothesis that *P. penetrans*, one of the most damaging *Pratylenchus* species, is pathogenic to soybean. Each year, two fields planted with the same soybean cultivar in a field with Plainfield loamy sand soil were studied. The fields were naturally infested with *P. penetrans* and the distribution of nematode population densities was highly variable due to previous research in the fields. At 6 to 11 days after planting, 25 (2009) or 16 (2010) randomly selected sites 5-m long in the soybean row were marked and numbered within each field. Soil samples were collected away from the row to estimate nematode inoculum levels. Two plants from the

ends of each marked site, collected on that and seven additional dates, were incubated for nematodes on Baermann funnels. A 1-meter section of plants in the middle of each site was harvested for soybean yield. Simple linerar regression was used to determine the relationship of grain yield to population densities of *P. penetrans* for each sampling date within a year, combining data from the two fields in the analysis. Initial population densities ranged from 0 to 374 (mean = 116) *P. penetrans* per 100 $cm^3$ soil in 2009 and from 0 to 1085 (mean = 212) *P. penetrans* per 100 $cm^3$ soil in 2010. For an initial population density of 100 *P. penetrans* per 100 $cm^3$ soil the predicted yield loss was 18% in 2009 ($P = 0.03$, $r^2 = 0.09$) and 25% in 2010 ($P = 0.001$, $r^2 = 0.37$). Nematode densities in roots were negatively related to soybean yield for four dates in 2009 with plants at the V1 to the R3 growth stage and on seven dates in 2010 with plants from the VE to R5 growth stage. The rate of nematode infection in roots peaked at about 500 growing degree days after planting and soil samples collected at harvest demonstrated soybean was a good host for nematode reproduction. This study indicates that *P. penetrans* can be a serious pest of soybean and should be included in nematode management plans for sandy soils.

EFFECT OF *XANTHOMONAS AXONOPODIS PV.DIEFFENBACHIAE* IN *ANTHURIUM ANDRAENUM* INFECTED WITH *RADOPHOLUS SIMILIS*. **Makimoto, Yoshimi and B. Sipes.** Dept. of Plant and Environmental Protection Sciences, University of Hawaii, 3190 Maile Way, Honolulu, HI 96822.

*Anthurium andraenum* (anthurium) is an important floriculture crop worldwide. A disease complex occurs in anthurium where the plants are infected with *Radopholus similis* and *Xanthomonas axonopodis* pv. *dieffenbachiae (Xad),* the causal agent of bacterial blight. The effect of *Xad* on anthurium and *R. similis* infection were evaluated in a factorial design experiment. The treatments included i) a foliar spray of $10^7$ CFU *Xad* per plant and inoculation with 50 *R. similis*, ii) inoculation with 50 *R. similis* only, iii) inoculation with $10^7$ CFU *Xad* only and iv) an untreated control. Six months after inoculation, anthuriums with *Xad* had less (Rf=0.61) reproduction of *R. similis* than the *R. similis* only treatment (Rf=1.02). The *Xad* and *R. similis* co-inoculation resulted in the smallest anthurium plants at harvest (7.07g) compared to the *Xad* only which had the largest plants (10.65g). The *Xad* only were also the tallest and had the greatest number of leaves. The *Xad* density in the *Xad* only treatment ranged between $8.3x10^5$ and $9.5x10^8$ CFU/ml/g and *Xad* and *R. similis* co-inoculation ranged between $2.8x10^5$ and $1.0x10^9$ CFU/ml/g at the termination of the experiment. Over the 6 month period, *Xad* inoculation may have suppressed the *R. similis* population. *Xad* inoculation improved plant weight but did not increase the incidence of anthurium blight. This effect is possibly due to poor incubation after the initial *Xad* inoculation leading to induced resistance with subsequence suppression of *R. similis* and stimulation of plant growth.

*POCHONIA CHLAMYDOSPORIA*: ADVANCES AND CHALLENGES TO IMPROVE ITS PERFORMANCE AS A BIOLOGICAL CONTROL AGENT OF SEDENTARY ENDO-PARASITIC NEMATODES. **Manzanilla-López[1], Rosa H., I. Esteves[2], M. Finetti-Sialer[3], P.R. Hirsch[1], E. Ward[1], J. Devonshire[1], L. Hidalgo[4], and B.R. Kerry[1].** [1]Plant Pathology and Microbiology Department, Rothamsted Research, Harpenden, Herts. AL5 2JQ, UK, [2]IMAR-CMA, of Life Sciences Dept., Faculty of Sciences and Technology, Coimbra, Portugal, [3]Consiglio Nazionale delle Ricerche, Istituto di Genetica Vegetale (CNR-IGV). Via Amendola 165/A, 70126, Bari, Italy, and [4]Centro Nacional de Sanidad Agropecuaria, Apartado 10, San José de las Lajas, Mayabeque, Cuba.

The nematophagous fungus *Pochonia chlamydosporia* (Clavicepitaceae) is one of the most studied biocontrol agents of plant endo-parasitic nematodes. The fungus is a parasite of nematode and mollusc eggs but occurs as a saprophyte in soils. In the rhizosphere, it can colonize the roots of host plants as some *Pochonia* species have an endophytic behaviour that may be beneficial to the host plant's defence against other soil borne pathogens. Several factors have contributed to target this fungus for nematode biocontrol. They include the saprotrophic behaviour in soil in the absence of both plant and nematode hosts, its worldwide distribution, natural occurrence in nematode suppressive soils, laboratory culturing, access to isolates (fungal collections) and effectiveness in nematode control. These factors, and the need to find alternatives to nematicides, have lead to the screening and testing of numerous isolates to find potential biocontrol agents against *Heterodera, Globodera, Meloidogyne, Nacobbus* and *Rotylenchulus* as shown by an increasing number of *Pochonia* native isolates reported. However, only a few isolates are commercially available. Facilities and resources for fungal production can be basic and not always subjected to regulatory mechanisms, which may limit their use and distribution. Interactions between plant-fungus-nematode are complex and, in order to exploit the fungus effectively in regulating plant endo-parasitic nematodes, a careful selection of fungal isolates (biotypes) appropriate for both host plant and nematode is essential. Research has covered so far a wide range of classic and molecular tools to address different aspects, including biology, genetic diversity, ecology of the tri-trophic interactions, and the effect of nutrition on key enzymes (e.g., VCP1) involved in the switch from the saprophytic to the parasitic phase of the fungus. Isolation and characterization of compounds related to fungal pathogenicity include pochonins, aurovertin-type metabolites and aromatic compounds. Although fungal chlamydospore-based products for application to soil have been

shown to be commercially viable, there is a need to reduce application dosages, optimise production and formulation methods, as well as to conduct more validation field tests. Genome sequencing and other -omics technologies will open new research avenues for *P. chlamydosporia* including discovery of new genes involved in the host-parasite interaction. It is expected that a better understanding of the biology, ecology and fungus-nematode interactions at the molecular level will improve genetic and chemical interactions that could lead to a more efficient and effective use of this biocontrol agent.

EFFECTS OF *CROTALARIA JUNCEA* ON THE ANHYDROBIOTIC STAGE OF *ROTYLENCHULUS RENIFORMIS*. **Marahatta, Sharadchandra P., K.-H. Wang, and B. S. Sipes.** Department of Plant and Environmental Protection Sciences, University of Hawaii, 3050 Maile Way, Honolulu, HI 96822.

Reniform nematode, *Rotylenchulus reniformis*, is a difficult nematode to control as it can enter into an anhydrobiotic stage to survive unfavorable conditions. Although sunn hemp, *Crotalaria juncea*, is known to produce allelopathic compounds against reniform nematodes, it is unclear if this allelopathic effect will suppress the anhydrobiotic stage of this nematode. Soils from a fallow pineapple field with a history of reniform nematode infestation were potted and conditioned by 1) keeping the soil dry (DRY), 2) irrigated (IRR), 3) growing sunn hemp (SH), and 4) growing cowpea (CP) for 3 mon. The conditioning created different regimes which encouraged the reniform nematode population together an anhydrobiotic stage. At the end of the conditioning, soils were either amended (SH+) or not amended (SH-) with sunn hemp foliage at 1% (w/w) in powdered form. Three cowpeas were seeded in each pot and grown for 3 wk to test for viability of reniform nematode. At termination of conditioning, DRY resulted in higher number of non-active coiled nematodes (anhydrobiotic stages) compared to SH and IRR ($P < 0.05$). At termination of the cowpea bioassay, SH+ consistently suppressed ($P < 0.05$) the number of reniform nematodes in soils conditioned with CP, but not in soil with DRY conditioning. Sunn hemp amendment only slightly reduced ($P > 0.05$) population densities of reniform nematodes in the soil condition with IRR or SH at termination of cowpea biossay. However, SH+ did reduce the number of reniform nematodes infecting bioassay cowpea roots in soils conditioned with SH or CP ($P < 0.05$). The effect of SH+ against reniform nematode infection on cowpea roots was not significant in IRR soil and was undetectable in DRY soil. Thus, SH+ did not suppress reniform nematodes efficiently in their anhydrobiotic stage but did suppress the nematodes in their active stages.

SUPPRESSION OF ROOT-KNOT NEMATODE DEVELOPMENT INDUCED BY COLONIZATION OF THE ENDORHIZA OF TOMATO WITH MUTUALIST ENDOPHYTIC MICROORGANISMS. **Martinuz, Alfonso[1*] and R. A. Sikora[1].** [1]INRES-Plant Protection, Soil-ecosystem Phytopathology and Nematology, University of Bonn, Nussallee 9, 53115 Bonn, Germany.*Corresponding author: martinuz@catie.ac.cr

The endophytic fungus *Fusarium oxysporum* strain Fo162 and the endophytic bacterium *Rhizobium etli* strain G12 have been shown to enhance plant resistance towards the root-knot nematode *Meloidogyne incognita*. The inoculation of tomato seedlings with these antagonists lead to significant reductions in the number of juveniles that penetrated the root and ultimately the number of galls and egg masses produced. The present study determined the influence of *F. oxysporum* and *R. etli* root colonization on juvenile development over time following synchronized nematode inoculation. The results showed that 14 and 21 days after nematode inoculation the rate of development to the 3$^{rd}$ stage juvenile as well as to the adult stage was significantly lower, 54 and 65 percent, in endophyte treated plants when compared to the untreated control over time, respectively. In addition, fungal and bacterial endophyte treatment led to a significant reduction in the number of eggs per female 35 days after nematode inoculation. The results demonstrated that *F. oxysporum* and *R. etli* root colonization restrain *M. incognita* development when the two organisms are present in the same root system. The mechanism of action is considered to be associated with reduction in giant cells formation and activity due to competition for nutrients in the endorhiza.

REACTION OF CERTAIN COMMON BEAN CULTIVARS TO ROOT-KNOT NEMATODE MELOIDOGYNE INCOGNITA IN ISMAILIA, EGYPT. **Massoud[1], Samia I. and S.H.A. Hassan[2].** [1]Dept. of Botany, Fac. of Agriculture, Suez Canal University; [2]Plant Pathology Researches Institute, ARC.

The species of Meloidogyne which was found most prevalent attacking common bean Phaseolus vulgaris in Ismailia Governorate ,was identified as M.incognita by using perineal pattern technique . This identification was confirmed by advanced methods, Isozyme technique. The reaction of five common bean cultivars to M.incognita showed that nematode was developed and reproduced well on all the tested cultivars with significantly differences in number of J2,gall numbers/plant and the number of egg masses / plant between them. The two cultivars Paulista and Xera were highly susceptible followed by Swiss Blane, while Bronco and Nebraska rated as moderate or less susceptible comparing with the three other cvs . Results of using Protein Electrophoresis analysis ( SDS-PAGE )

indicated differences in buffer soluble protein extracted from the leaves of the five tested bean cultivars in relation to infection with M.incognita.The differences in protein banding profile are expectable due to the differences in genetic composition on these cultivars. The differences in protein banding patterns among healthy and infected samples proved the difference in response of those cvs. to the infection. Growth response of the tested cultivars to the M. incongnita infection had a negative effect on plant growth, number of nodules and pods weights. Chlorophyll content in the leaves of paulista cv. in response to M. incognita infection was decreased significantly during different ages of plant development comparing with the control plants.

ROTATIONS AND SOIL NEMATODE POPULATIONS. **M. M. Matute[1] and A. Anders[2].** [1]Dept. of Biology, University of Arkansas, 1200 North University Drive, Pine Bluff, Arkansas 71601, [2]Rice Research and Extension Center, Stuttgart, Arkansas 72160.

Crop rotation sequences that will reduce populations of plant feeding nematodes and increase populations of decomposer nematode guilds are desirable. We investigated the influence of crop rotation sequences on soil nematode populations in the rice agroecology of Stuttgart, Arkansas. Tillage versus non-tillage treatments were applied to the crop rotation sequence in a split-plot design. Rotated crops were *Oryza sativa* (rice), *Glycine max* (soybean), and *Zea mays* (corn/maize) in the following combinations: fallow-fallow-fallow, rice-rice-rice, rice-rice-soybean, soybean-soybean-rice, rice-rice-corn, corn-corn-rice, rice-corn-soybean, and rice-soybean-corn. Samples were collected at plant-establishment and at harvest during the third crop. Nematodes recovered were categorized according to their families, trophic groups, and colonizer persister (cp) classes. Factors analyzed for their effects on nematode categories included, rotation (R), tillage (T), sampling time (ST), R x T, R x ST, T x ST, and R x T x ST. A total of 18 nematode families were recovered: bacterivores included Rhabditidae, Panagrolaimidae, Cephalobidae, Plectidae, and Prismatolaimidae; fungivores included Aphelenchidae and Aphelenchoididae; plant feeders included Tylenchidae, Psilenchidae, Hoplolaimidae, Meloidogynidae, Heteroderidae, Pratylenchidae, and Longidoridae; omnivores included Dorylaimidae, Thornenematidae and Qudsianematidae; and carnivores included Mononchidae. All rotations that included *G. max* significantly ($P<0.05$) or numerically increased soil nematode populations, irrespective of trophic groups, while all rotations that included *O. sativa* and *Z. mays,* had a nematode reductive effect.. For example, there were 428 bacterivorous nematodes per 100 mL soil in the R-C-S rotation compared to the 336 nematodes/100 mL soil for the R-R-R ($P=0.06$). Similarly, for the plant feeders the rotation combination R-C-S recorded 140 nematodes per 100 mL soil compared to 16 nematodes per100 mL soil for the R-R-R combination($P<0.001$). For the bacterivorous nematodes only the R x ST interaction was significant ($P<0.05$); populations were higher during plant-establishment and with rotations including soybean. Factors that significantly ($P<0.05$) affected populations of fungivores were ST, R, ST x R and ST x R x T, respectively; R-C-S and plant-establishment recorded the highest populations, etc. Factors R and ST x R significantly ($P<0.001$) affected mean numbers of plant-parasitic nematodes; higher populations of plant-parasitic nematodes were recorded at harvest than at plant establishment and also the R-R-R and R-C-S, respectively recorded the lowest and highest populations of these parasites. Tillage has previously been reported to stimulate the proliferation of fungivorous and bacterivorous nematodes. In this investigation tillage had no significant influence on plant-, bacterial- or fungal- feeding nematode populations but it was the only factor that significantly ($P<0.05$) affected populations of omnivorous nematodes. Higher populations levels of omnivores occurred in no-till than in tilled plots, corroborating earlier reports. Only the interaction T x ST had a significant effect on the populations of the carnivorous nematodes ($P<0.05$). That tillage alone did not significantly influence their numbers was not expected, considering that carnivores have been reported to be very sensitive to changes in their environment.

TIMING OF POST TREATMENT IRRIGATION IMPACTS THE NEMATICIDAL VALUE OF SPIROTETRAMAT. **McKenry, Michael, S. Kaku and T. Buzo.** Department of Nematology, University of California, Riverside, Riverside, CA 92521.

Ruby Seedless grapes, *Vitis vinifera*, were grown in open-bottom microplots 60 cm in diameter by 120 cm deep. After five years population levels of *Meloidogyne incognita* averaged 1100 J2 per 250 $cm^3$ soil sample. Vines were irrigated by drip and during the first two weeks after Movento spray they would normally receive 11.4 L of water/vine every three days. On April 28, 2009 150 vines received either a single foliar spray of Movento at 291 ml/ha, 457 ml/ha or were untreated. Penetrator, an adjuvant from Helena Chemical Company, was added to the spray at 237 ml/378 L water. Fruit from ten reps of each treatment were used for yield data collection while soil samples were collected monthly from each of five vines per replicate. Except for being one week later with the sprays, this experiment was repeated in 2010. Yield and nematode population reductions were quite similar for each of the two years except that 2010 yield improvement due to Movento was greater than that achieved in 2009. Irrigation timing was unaltered or made 3, 6, 9 and 12 days after the Movento spray. Soil samples following each treatment were collected from 0 to 45 cm depth at 30 day intervals for 6 months. Samples were extracted using a 45 micron

sieve followed by three days of mist extraction. Grapevines were harvested each year in September. Yields for 2009 were significantly improved by waiting 9 days before resumption of irrigation regardless of application rate. The only other timing that provided significant yield benefit was a 6-day wait when sprayed at the 291 ml/ha treatment rate. Yields for 2010 were significantly improved only after the 291 ml/ha treatment rate whether irrigation was resumed at 3, 6 or 9 days with greatest yield improvement occurring at 9 days. Nematode control data correlated with increased grape yield. Inattentiveness to irrigation timing resulted in lack of significant nematode control. Avoidance of irrigation for 9 days provided four months of significant nematode reduction in 2009 and 2010 while irrigation resumption at 0, 3, 6, and 12 days provided less consistent and generally less effective nematode reductions. Reduction of *Meloidogyne* spp populations by 50% for five to six months is possible with attentiveness to post treatment irrigation timing. In larger field trials it has been notable that greatest nematode reductions occur between drip emitter sites rather than at drip emitter sites.

TRENDS IN AUTHORS, NEMATODES, AND SUBJECT MATTER IN THE *JOURNAL OF NEMATOLOGY,*1969 – 2009. **McSorley, Robert.** Dept. of Entomology and Nematology, University of Florida, PO Box 110620, Gainesville, FL 32611-0620.

Issues of the Journal of Nematology from 1969-2009 were examined to determine trends in authorship and subject matter. Data were collected on authors, affiliations, locations, research funding, nematodes, and nematological subject matter. These variables were then compared among the 4 decades involved. Some of the more prominent changes noted included: a decrease in the number of papers published in the Journal of Nematology in the 1990s and 2000s from a peak in the 1980s; an increase in number of authors per paper in each decade; an increased percentage of international authors in the 1990s and 2000s compared to 1970s; and changing roles of the USDA and different states over a period of 4 decades. Plant-parasitic nematodes were the main organisms studied in 73.4% of all papers published the Journal of Nematology from 1969-2009, whereas studies of marine nematodes or vertebrate parasites were rare. The greatest changes in subject matter were increases in papers on biological control and resistance in the 1990s and 2000s compared to the 1970s and 1980s. Additional trends and subjects are discussed, and data are provided comparing differences among the 4 decades for various aspects of nematology.

THE DEVELOPMENT OF ENCLOSURE® (IPRODIONE) AS A NEMATICIDE FOR SELECTED US CROPS. **Meier, Deborah[1], A. R. Finlay[2], D. A. Jupp[3], L. Maertens[2], T. Bogaert[2].** [1]Devgen US Inc., 413 McFarlan Road, Suite B, Kennett Square, PA 19348, [2]Devgen nv, Technologiepark 30, 9052 Gent – Belgium, [3]Disa BioTechnologies cc, Postnet 327, Private bag X16, Constantia 7848, South Africa.

Devgen has developed a novel nematicide that protects numerous crops from damage by plant parasitic nematodes. Many of the traditional nematicides used in the US and other parts of the world have been or are in the process of being phased out due to regulatory concerns. New regulatory fumigant management plans (FMP) being implemented across the US are increasing grower costs. FMP also can impact yield potential if portions of the field are unprotected due to required buffer zones associated with water bodies or residential facilities. Devgen used its proprietary technology platform to screen a diverse group of products with favorable environmental profiles, to identify a non-fumigant nematicide. Several candidates were identified and patent applications were filed on the use of the selected compounds for nematode management. Studies with the lead candidate, iprodione, demonstrated activity on key nematode species in a wide range of crops. Crops attacked mainly by *Meloidogyne spp.*, have been the focus of regulatory and development trials in the US and EMEA. Submissions have been filed in US, Turkey, Spain, Italy, Greece, Morocco and South Africa. The lead candidate was granted regulatory approval in 2009 in Turkey (commercialized as DEVGUARD® 500SC on protected vegetable crops) and the US (commercialized as ENCLOSURE® 4 Flowable Fungicide and Nematicide on peanuts). ENCLOSURE was launched in 2010 for use in commercial peanut production. US registration is pending for use on fruiting vegetable and cucurbit crop groups. Key features of ENCLOSURE include proven efficacy and crop safety, CAUTION signal word, reduced risk to the environment and worker, flexibility in application timing, and no requirement for a special applicator certification or license. Attributes of ENCLOSURE make it ideal for integration into a nematode management program. Application in peanut can be made via soil treatment at planting and band treatment at key stages during the crop cycle. Proposed applications in the fruiting vegetable and cucurbit crops are pre-transplant/seeding through mechanical incorporation or drip chemigation plus post application(s) through drip chemigation. Activity is through contact. Laboratory studies with *Meloidogyne spp.* inoculated at periods up to 35 days after tomato transplanting indicate residual activity of 14 days at 1 kg ai/ha increases to 28 days at 3 kg ai/ha. ENCLOSURE can also extend nematode management when used following a pre or at planting nematicide. ENCLOSURE field efficacy studies have shown improved yields in peanuts, tomato, pepper, and cucurbit crops when nematode populations are low to moderate.

QUANTIFYING INFECTIVE *HETERODERA SCHACHTII* IN SOIL WITH A SIMPLE AND SENSITIVE BIOASSAY. **Meinecke[1], Annabell, A. Hermann[2], and A. Westphal[1*].** [1]Julius Kühn-Institut, Federal Research Centre for Cultivated Plants, Institute for Plant Protection in Field Crops and Grassland, Toppheideweg 88, 48161 Münster, Germany; [2]The Bavarian State Research Center for Agriculture, Institute for Plant Protection Nematology, Lange Point 10, 85354 Freising, Germany.

*Heterodera schachtii* is a major pathogen of sugar beet worldwide. Management options rely on wide crop rotations with non-hosts and use of resistant cover crops, and recently have been amended to include tolerant and resistant sugar beet cultivars. Implementing these strategies requires knowledge of the *H. schachtii* infestation levels in fields. Determining population densities is cumbersome when mixed nematode populations are present in production fields. After determining that radish roots are rarely penetrated by other plant-parasitic nematodes that may be present in sugar beet fields, e.g., *H. avenae, H. filipjevi, Globodera* spp. *Meloidogyne hapla* and *Pratylenchus penetrans*, a bioassay was standardized. Only the rarely occurring *M. incognita* penetrated at some level. Soil samples of 50 grams dry matter were single seeded with radish cultivar 'Saxa 3', and adjusted to uniform soil moisture in glass containers. After incubation for four days at 28/23 °C and 16/8h day/night cycle, radish roots were harvested, stained with acid fuchsin, and nematodes that had penetrated were counted under a microscope. When inoculated with proportional mixtures of *H. schachtii* and *H. avenae* or *H. filipjevi*, counts of sugar beet cyst nematodes were not impacted by the addition of the cereal cyst nematodes. In different soil types, the nematode penetration depended on inoculation density. In comparison to other methods, equivalent soil samples from different fields were analyzed by counting nematodes detected in the bioassay (BA), after cyst and egg extractions (EX), or in the acetox method (AC). Count correlations were: BA and EX, $R^2 = 0.6$; EX and AC, $R^2 = 0.5$; and BA and AC, $R^2 = 0.4$. The bioassay was a time-saving and accurate method. Only infectious J2 of *H. schachtii* were recognized, thus eliminating the subjective distinction into healthy and diseased eggs as necessary in the cyst and egg extraction methods. The bioassay is a fast, simple and accurate method for determining *H. schachtii* infestation densities in soil, and could become part of integrated nematode management systems.

HOW MUCH DO SOIL CONDITIONS CONTRIBUTE TO NEMATODE PARASITIC VARIABILITY? **Melakeberhan, Haddish[1], S. Mennan[2], A. Kravchenko[3], J, Dahl[3] and D. Warncke[3].** [1]Dept. of Horticulture, Michigan State Univ. East Lansing, MI 48824 USA, [2]Dept. of Crop Protection, Ondokuz Mayis Univ. Samsun, Turkey 55139, [3]Dept. of Crop and Soil Sciences, Michigan State Univ. East Lansing, MI 48824.

Nematode parasitic variability is a major challenge in modern agriculture. Most of our efforts to understanding the host-parasite interactions and developing management strategies have focused on the nematode and plant species in question. Our understanding whether or not and to what extent soil conditions influence nematode parasitic variability has been limited. Since soils are heterogeneous and production practices involve a range of inputs, it is worth considering if and how soil conditions influence nematode parasitic variability. In separate greenhouse studies, populations of *Meloidogyne hapla* (NRKN) and *Heterodera glycines* (SCN), both with known parasitic (genetic) variability complexes, and different soil types, and soil amendments were used to test *the hypothesis that nematode populations with variable parasitism traits respond differently to soil amendments in the same soil type.* The NKRN studies included populations from Rhode Island, Connecticut, New York, and Michigan inoculated into tomato grown in five soil types (pH 4.5 to 8.0) amended with N-Viro Soil (NVS), an alkaline stabilized biosolid product with soil nutrition enrichment capacity and potential for suppressing nematodes. Across experiments, the effect of NVS on the *M. hapla* populations varied. The most consistent effects observed were that of soil type and two-way interactions to affect nematode population dynamics and plant growth, suggesting that NVS application is likely to be site-specific. The SCN studies included SCN populations, soybeans, and mineral soils amended with compost and commercial fertilizer. Interaction effects of SCN populations*amendment, SCN*soil type, amendment*soil type and/or SCN*amendment*soil type were statistically significant, proving the hypothesis true. The studies suggest that soil conditions may be influencing nematode parasitic variability and provide a *proof-of-concept* for: ***i***) understanding the basic soil-driven bio-ecological conditions that may influence nematode adaptation and parasitic variability, and ***ii***) developing applied multi-factor site-specific management practices.

*BRADYNEMA LISTRONOTUM*, A NEW NEMATODE PARASITE OF THE CARROT WEEVIL *LISTRONOTUS OREGONENSIS*. **Mimee, B., G. Bélair, and G. Boivin,** Horticulture Research and Development Centre, Agriculture & Agri-Food Canada, Saint-Jean-sur-Richelieu (Quebec) Canada J3B 3E6.

The nematode *Bradynema listronotum* (Nematoda: Allantonematidae) is an entomopathogenic nematode recently described from infected specimens of carrot weevil adults (*Listronotus oregonensis*). In southwestern Quebec, infection levels ranged from 20 to 60%. All larval stages and adults of the nematode are found in the insect hemocoele. The nematode feeds on the reproductive system of both sexes and infected females soon stopped

laying eggs but survived for several weeks. The emergence of *B. listronotum* juveniles from infected adult weevils was continuous with an average of 5,500 juveniles emerging over a period of 45 days. The infected female weevils, although sterile, nonetheless attempted to oviposit and, doing so, deposited nematode juveniles at the oviposition site. Because other uninfected females are likely to use the same site for oviposition, this behavior contributes to the spreading of the nematode. Infected males were infertile but did not transmit nematodes during mating. It was possible to infect the larvae, pupae and adult weevils in the laboratory. The distribution of this nematode in southwestern Quebec appears to be limited geographically. Further research is needed before conclusions can be drawn regarding the feasibility of using B. *listronotum* to control carrot weevil.

BIOLOGICAL CONTROL OF IMPORTED CABBAGEWORM (*PIERIS RAPAE*) WITH THE ENTOMOPATHOGENIC NEMATODE (EPN) *STEINERNEMA FELTIAE* MG 14. **Mohammed R., Rafid H. and B. S. Sipes.** Plant and Environmental Protection Sciences, University of Hawaii at Manoa, Honolulu, USA 96822.

Imported cabbageworm (*Pieris rapae* L.) is an economically important pest on cabbage plants. Losses can be up to 71% of yield. EPNs have been used to control *P. rapae* but in population densities of millions of EPNs/ha. Our hypothesis is that EPNs might be used at lower population densities and still provide effective control, similar to that achieved with high IJ population densities. Ten 4$^{th}$ instar larvae of *P. rapae* were placed in a 64 cm$^2$ Petri dish with 4, 8, 12, 16, or 20 *S. feltiae* MG 14/5 cm$^2$ for 36 hr. The final insect death was recorded 48 hr after removing the surviving larvae to an EPN-free dished. Larvae mortality averaged 78%, 92%, 94%, 96%, and 100% at 4, 8, 12, 16, and 20 EPN/5 cm$^2$ respectively. Data were analyzed by regression and gave a linear model where $y = 7.76 + 0.12(x)$, ($P$=0.05, $r^2$=0.82) where y is mortality and x is EPN/5cm$^2$. EPN population densities as low as 20 or 16/5cm$^2$ can be effective in control of *P. rapae* in the laboratory. The regression model explained the relationship between *P. rapae* larvae mortality and the efficacy of *S. feltiae* MG 14 at low population densities.

AN INDIAN ISOLATE OF THE BACTERIUM *PASTEURIA* PARASITIC TO THE PIGEON PEA CYST NEMATODE *HETERODERA CAJANI,* ALSO INFECTS THE POTATO CYST NEMATODE *GLOBODERA PALLID*A FROM THE UK. **Mohan[1,2], Sharad, J. Rowe[1], T.H. Mauchline[1], K.G. Davies[1].** [1]Plant Pathology and Microbiology, Rothamsted Research, Harpenden, Herts. AL5 2JQ, United Kingdom and [2]Affiliation Author: Division of Nematology, Indian Agricultural Research Institute, Pusa Campus, New Delhi.110012, India.

The *Pasteuria* group of bacteria are Gram positive, endospore-forming parasites and are currently being developed into biological control agents for the control of plant-parasitic nematodes on golf courses. One of the major challenges is to control agricultural pest nematodes in field situations where alternatives to nematicides are being sought. We described a population of *Pasteuria* isolated from the pigeon pea cyst nematode, *Heterodera cajani,* from India which multiplied equally well on the potato cyst nematode *Globodera pallida* from the UK. Although the number of spores adhering to second-stage juveniles (J2) of *H. cajani* (7.3 spores J2$^{-1}$) and those to *G. pallida* (6.7 spores J2$^{-1}$) showed no statistical difference, the orientation of endospore attachment differed with a great number of endospores attached to the cuticle of *G pallida* by their convex surface. The inverted endospores germinating on the juveniles of *H. cajani* were significantly fewer in number (13%) than those germinating on the juveniles of *G. pallida* (22%). Within six days of spore attachment, the inverted endospore constituted only of the exosporium as the central body collapsed leaving behind a depression, which appeared to have been plugged and indicated germination in the J2s preceding their penetration of the host plant. Both the inverted and the conventionally attached endospores, produced -bacillus-like rods that completed their life cycle in less than 15 weeks within the females of *G. pallida*. The endospore size (5.28μm ± 0.5) and a 1,430 base pair fragment of the 16S rRNA gene sequence of HcP showed 98.6% relatedness to the orthologous gene in *P. nishizawae,* suggesting phylogenic similarity. However, the two differed in their ability to effect cross-generic attachment to variable hosts from different geographical regions. As such, it is contentious whether both belong to the same species.

NOVEL GENOME STRUCTURES FOR DIVERSE MITOCHONDRIAL GENOMES. **Morris, Krystalynne[1], W. K. Thomas[1], P. Schiffer[2], S. Lewis[3], B.C. Hyman[3], I.T. De Ley[3], P. De Ley[3], A. Holovachov[4].** [1]Hubbard Center for Genome Studies, University of New Hampshire, 35 Colovos Road, 4$^{th}$ Floor Gregg Hall, Durham, NH 03824. [2]University of Köln, Zoologisches Institut, Zülpicher Strasse 47b, 50674 Köln, [3]University of California-Riverside, Webber Hall, Riverside, CA 92421, [4]Swedish Museum of Natural History,Department of Invertebrate Zoology, Box 50007, SE-104 05 Stockholm, SWEDEN.

Mitochondrial genomes of metazoa are highly conserved in gene content allowing for their comparison across extremely divergent organisms. This genome can be a source of important and useful markers of evolutionary history within and between closely related species, especially when coupled with PCR-based sequencing approaches. Furthermore, the re-arrangement of genes in these genomes provides opportunities to understand the mechanisms

of recombination. However, the paucity of diverse mtDNA genomes has limited the development of universal primers. Having reference genomes of diverse nematodes will support informed primer design across the phylum. To examine the diversity of mitochondrial genome sequences and gene orders, we assembled the mitochondrial genomes from five newly sequenced nematode genomes representing divergent lineages. These genomes revealed both conserved and divergent patterns of mitochondrial genome evolution, furthering our understanding of mitochondrial gene evolution in nematodes and providing reference genomes for intra-specific analysis.

THE GENOME OF A SINGLE NEMATODE. **Morris, Krystalynne[1], H.M. Bik[1], and W. K. Thomas[1*].** [1]Hubbard Center for Genome Studies, University of New Hampshire, 35 Colovos Road, 4th Floor Gregg Hall, Durham, NH 03824.

The phylum Nematoda is hyperdiverse and nematodes perform critical ecosystem functions in virtually all environments. To contribute to our understanding of nematode biology, nematode genomes are being generated for a large number of species. These genomes provide critical information on the evolutionary history and functional diversity of this group. One of the major obstacles to understanding the phylogenetic and functional diversity of nematodes is the fact that the vast majority of species are unculturable. Recent, advances in PCR and DNA sequencing have made it possible to amplify and sequence an organism with a small number of cells. To test the approach for sequencing a single nematode, we isolated an individual nematode from a marine sediment core and identified the nematode based on morphology to genus-level (*Oncholaimus).* DNA extracted from this nematode was subjected to whole genome amplification augmented with Trehalose. This prepared template was submitted for Illumina paired-end sequencing. From this source, 48,885,973 reads were generated, and assembled into 160,757 contigs with 92,329,785 bases in those contigs. *Ab initio* gene predictors and algorithms are being used to predict a catalog of genes in this assembly. The vast majority of contigs are consistent with a nematode origin including assembly of the predicted rRNA genes, and mitochondrial genome. The quality of the assembly is assessed using a standard set of orthologous genes expected in any nematode species.

A HIGHLY PATHOGENIC ROOT KNOT POPULATION (*MELOIDOGYNE* SP.) OF PEPPER IN SINALOA, MEXICO. **Mundo-Ocampo, M.[1,2], J. R. Camacho-Baez[2] J.G. Baldwin[1], A. R. Armenta-Bojorquez[2], T. Pereira[1] M.A. Apodaca-Sanchez[3], and P. Castaneda- Ibarra[4].** [1]Department of Nematology, University of California-Riverside, [2]CIIDIR-IPN-Unidad Sinaloa, Guasave, Sin, Mex. and [3]Escuela Superior de Agricultura, Universidad Autónoma de Sinaloa.

A considerable acreage of several cultivars of chile peppers (*Capsicum spp.*), including Jalapeño, Anaheim, Bell, Ancho, Serrano, Caribe, and others are grown in the Northern region of Sinaloa, Mexico. Crop production is mainly used for local consumption, industrial processing and export. Most fields are cultivated under drip irrigation and some under greenhouse or plastic tunnels conditions. During a survey supported by UCMEXUS/CONACYT, farmers indicated incidence of soil borne diseases in pepper fields. A few months after crop establishment plants showed symptoms of stunting, chlorosis, wilting and often plants died. Close inspection of the roots showed severe galling associated with nematodes and vascular discoloration probably due to *Fusarium oxysporum.* Other soil fungi/nematode interactions were also observed. When galls were dissected females of *Meloidogyne sp.* were evident. Morphological observations of females, juveniles and males, including light microscopy and scanning electron microscopy did not match morphological characters of any root knot species known to be commonly present in the region (*M. incognita, M. javanica* or *M. arenaria).* Enzyme profiles, Esterase (EST) and Malate dehydrogenase (MDH) as well as molecular sequencing analyses suitable for diagnosis are in progress. Possible species identity and possible origin to this region is discussed.

EVALUATING HATCHING FACTORS OF *GLOBODERA PALLIDA* AND *TABACUM.* **Navarre[1,2], Roy., S. Kumar[2], and R. Zemetra[3].** [1]USDA-ARS, Prosser, WA 99350 [2]Washington State University, Prosser, WA. 99350 [3]University of Idaho, Moscow ID.

*Globodera pallida* was found in southern Idaho in 2006 and an eradication effort is underway. PCN can persist for decades in fields in the absence of a host and is a formidable pest of potatoes largely for this reason. Cysts contain hundreds of eggs that hatch in response to unidentified compounds called hatching factors (HFs) that are present only in root exudates from potatoes and closely related plants. We are trying to identify HFs and trap crops that can be used to induce a "suicide hatch" in the absence of a host. Partial purification by HPLC suggests over 10 compounds are present with hatching activity. The activity elution profile is not consistent with glycoalkaloids being major contributors of hatching activity. *S. sisymbriifolium* was found to induce hatching of the Idaho population. To explore whether a more extensive root system would increase the efficacy of a trap crop, *S. sisymbriifolium* was then transformed with the rol genes to promote root growth and potentially up-regulate secondary metabolism. In addition, over 100 non-*Solanaceous* plants were evaluated for hatching activity.

ALTERNATIVES TO METHYL BROMIDE SOIL FUMIGATION FOR NEMATODE CONTROL IN FLORIDA STRAWBERRY. **Noling[1], Joseph W. and M. Cody[1]**, [1]University of Florida, IFAS, Citrus Research and Education Center, Lake Alfred, FL 33850.

This USDA ARS funded project was conducted during 2006-2011 to demonstrate and improve the performance and consistency of next-best chemical alternatives to methyl bromide in large scale, grower field demonstration trials in Florida strawberry. Alternative chemicals evaluated included individual and or combined uses of methyl bromide, methyl iodide, chloropicrin, dimethyl disulfide, and 1, 3-dichloropropene (1,3-d). A variety of drip fumigants were also evaluated for pest control efficacy and strawberry yield. Secondary objectives were to evaluate the feasibility of using two drip tapes per bed rather than one to enhance efficacy and yield of methyl bromide chloropicrin and drip applied fumigants; and use of a high barrier, gas impermeable mulch film to reduce emissions and soil fumigant application rates. Assessments of pest control, plant size, health, and vigor were periodically made to characterize treatment differences. Strawberry fruit were commercial hand harvested at many farm locations on a 2 to 3 day basis from early December generally through March or April. Relative strawberry yields were also determined from ground truth survey of plant size categories and with NDVI (Normalized Difference Vegetation Index) using GreenSeeker® (NTech Industries; Ukiah, Ca) optical sensors. All treatments were arranged at their respective farm sites as a completely randomized block design with 4 replications per treatment. Plot sizes varied from 2 to 12 rows or 0.06 to 0.4 acres among the different farm locations. In some studies, fumigant gas concentrations were measured with a MiniRae® 2000, portable VOC meter and or with GasTek® Trichloroethylene colorimetric detector tubes under a variety of plastic mulch films of differing permeability, at different locations across the mulch covered plant bed to characterize soil atmosphere gas concentrations, retention characteristics of different plastic mulches over time, as well as relative differences in cross bed, gas phase movement of the different fumigants with time. In general the results of monitoring soil air concentrations was able to confirm and differentiate, relative to standard low density polyethylene mulch, broad categories of gas impermeability among plastic mulches to different fumigants. In these trials, most alternative fumigants evaluated produced yields which were statistically equivalent to that of methyl bromide chloropicrin. Not all coformulated fumigants of chloropicrin and 1, 3-d proved to be effective for maintaining strawberry crop productivity. Strawberry plant growth and yield was usually significantly improved 10 to 15% when drip fumigants were delivered with two drip tapes per bed compared to one. With two drip tapes per bed, improvements in strawberry plant growth occurred as a result of a horticultural effect (improved water and nutrition) and also as a fumigation effect (improved movement and bed distribution of the fumigant). Relative strawberry yields determined from ground truth survey of plant size categories was well correlated with NDVI estimates of canopy cover using Greenseeker optical sensors of strawberry plant reflectance, and to commercially hand harvested small plot yields.

MANAGING ROOT-KNOT NEMATODES: A CASE FOR COVER CROPS IN ESTABLISHING PEACH ORCHARDS. **Nyczepir[1], Andrew P. and J. Cook[2].** [1]USDA-ARS, SE Fruit & Tree Nut Research Laboratory, 21 Dunbar Road, Byron, GA 31008 and [2]University of Georgia Cooperative Extension Service, Butler, GA 31006.

Root-knot nematodes (*Meloidogyne* spp.) are an important pathogen of peach in the United States. Several *Meloidogyne* spp. have been reported to cause damage to stone fruits, but *M. incognita* and *M. javanica* are the predominant species on peach. Preplant fumigant nematicides have traditionally been used to control *Meloidogyne* spp. in peach in the southeastern United States. In recent years growers have faced economic hardships, making it difficult to afford costs associated with these chemicals that can be hazardous to humans and the environment. Finding an alternative to chemical control of root-knot nematode is warranted. Previous greenhouse studies indicated Jesup (Max-Q) tall fescue grass to be a nonhost for *M. incognita* and a poor host for *M. javanica*. In another greenhouse study, penetration, development, and reproduction of *M. incognita* in Jesup (Max-Q) and Rutgers (susceptible) tomato seedlings were also studied. Each seedling was inoculated with approximately 5,000 eggs per pot. Seven days later the entire seedling was bare-rooted and transplanted into a new pot. Seedlings were harvested at 7, 14, 21, and 28 days after transplanting. The root systems were stained and then evaluated for numbers of J2 in roots and stage of development on each harvest date. Results indicated that *M. incognita* J2 penetrated tall fescue roots, but that the majority of the nematodes failed to mature and reproduce. In 2005, a field experiment in Georgia was initiated to study the effects of 1- and 2-yr tall fescue preplant rotations for the management of *M. incognita*. Both tall fescue rotations and preplant Telone II® fumigation suppressed ($P \leq 0.05$) population densities of *M. incognita* J2 in soil compared with nonfumigated plots prior to orchard establishment in 2009. In 2011, tree growth was greatest in fumigated and 2-yr tall fescue rotation plots, intermediate in 1-yr tall fescue rotation plots and lowest in nonfumigated plots. These results provide insights into the possible use of tall fescue as an alternative to preplant chemical control of *M. incognita* in peach.

CHARACTERIZATION AND PHYLOGENETIC RELATIONSHIPS OF A NEW *PHOTORHABDUS LUMINESCENS* SUBSPECIES (GAMMA-PROTEOBACTERIA: ENTEROBACTERIACEAE), THE SYMBIONT OF *HETERORHABDITIS SONORENSIS* (NEMATODA: HETERORHABDITIDAE). **Orozco, Rousel A. and S. P. Stock.** Department of Entomology, University of Arizona. Forbes Bldg. Rm 410. 1140 E. South Campus Dr., Tucson, Arizona, USA.

*Photorhabdus* are motile Gram-negative bacteria that have a mutualistic association with *Heterorhabditis* nematodes (Heterorhabditidae). These bacteria possess peculiar biochemical characteristics such as inability to reduce nitrates, and the capacity to ferment only a limited number of carbohydrates. *Heterorhabditis* nematodes vector the bacteria from one insect host to another and also provide shelter to the bacteria from soil stressors and antagonists. Once inside the insect host, the bacterial symbionts are released and produce toxins and secondary metabolites and broad spectrum antibiotics, that kill that the host by septicemia within 48 hrs. At present, three *Photorhabdus* spp. have been identified: *P. luminescens, P. temperata* and *P. asymbiotica*, and many subspecies for each taxon have also been described. Characterization of new species and subspecies has been based on sequence data, mostly 16SrDNA gene, and also on a selection of protein coding genes. In addition to this, phenotypic traits including temperature growth, colony morphology, color, light production, carbohydrate response and assimilation have been considered. In this study, we characterize the bacterial symbiont of *Heterorhabditis sonorensis,* a recently discovered entomopathogenic nematode species form the Sonoran desert in Arizona. We considered a selection of classic biochemical and molecular methods including sequence data from six genes: 16s rDNA, and five protein coding genes: *ser*C, *gyr*B, recA, *glt*X, *dna*N. Evolutionary relationships of this new *Photorhabdus* subsp. was inferred considering maximum parsimony and Bayesian analyses. Results from this study indicate this bacterium has a maximum temperature growth in nutrient broth at 37 °C. Cells are Gram-negative, motile, oxidase negative, catalase, arginine dihydrolase and gelatinase positive. This subspecies reacts negatively to m-inositol, formic, propionic, galacturonic and citric acid . But it reacts positively to D-alanine. Phase I colonies are bioluminescent, granulated, convex and opaque and have a sticky consistency. Phylogenetic analyses indicate this subspecies belongs to the *luminescens* group and has *P. luminescens luminescens* as their sister taxa.

EDAPHIC FACTORS INVOLVED IN THE DELINEATION OF MANAGEMENT ZONES FOR *ROTYLENCHULUS RENIFORMIS*. **Overstreet[1], Charles, E.C. McGawley[1], D. Burns[2], and R.L. Frazier[3].** LSU Agricultural Center: [1]Dept. of Plant Pathology and Crop Physiology, 302 Life Science Bld., Baton Rouge, LA 70803, [2]County Agent, 107-A Arts Drive, St. Joseph, LA, 71366 and [3]County Agent, 114 North Cedar Street, Tallulah, LA 71282.

The reniform nematode (*Rotylenchulus reniformis*) is a serious pest of cotton in the alluvial soils along the Mississippi River in Louisiana. Fumigants have proven successful in reducing losses from this nematode but may not be required throughout the entire field due to soil texture differences. A two-year study was initiated in 2009 in a production field heavily infested with reniform nematode and which there were marked differences in soil type to evaluate the areas that would respond to the application of a fumigant. The field was divided into five zones based on apparent electrical conductivity ($EC_a$). Four verification strips (16 rows each of 1,3-dichloropropene at 28 l/ha and untreated rows through the length of the field) were applied in the field through four of the five zones. The fifth zone was very heavy clay with limited distribution in the field. The greatest response (147 kg/ha of lint) to the fumigant occurred in the soil zone with the lowest $EC_{a\text{-}dp}$ reading (34.3-59.0 mS/m). Soil samples were collected for nematode, nutrient, and texture analysis through the soil profile in 15 cm increments to a depth of 60 cm from sampling sites within zones and verification strips. Of the nutrients analyzed (P, K, Mg, Ca, S, and Zn) P and Zn were low across all the soil zones, S in the three with the lowest $EC_{a\text{-}dp}$ reading, K and Ca were low in the two soil zones with the lowest $EC_{a\text{-}dp}$ readings, and Mg in only the lowest $EC_a$ zone. Soil pH was similar through the profile in all the zones. Clay content ranged from 6-29% through the zones and profile with the least amount found in the two lowest $EC_a$ zones. Sand content declined as the $EC_a$ reading increased but was similar for the two zones with the highest $EC_a$ readings. Reniform nematode populations were significantly different among treatments and soil zones but occurred in very high levels in zones with high $EC_a$. Higher levels of several nutrients were found in the zone with the least response to the fumigant. However, the positive response to fumigation for reniform nematode appears to occur in soils with much higher clay content than has been found for other cotton nematodes such as *Meloidogyne incognita*.

NEMATOPHAGOUS FUNGI, TEMPERATURE EFFECTS, AND THE DYNAMICS OF INTERSPECIFIC COMPETITION. **Pathak[1], Ekta, F. E. El-Borai[1,2], R. Campos-Herrera[1,3], R.J. Stuart[1] and L.W. Duncan[1].** [1]Citrus Research and Education Center, University of Florida, Lake Alfred Fl 33850, [2]Plant Protection Dept. Faculty of Agriculture, Zagazig University, Egypt and [3]Instituto de Ciencias Agrarias, CSIC, Serrano 115 dpdo Madrid, 28006 (Spain).

Nematophagous fungi (NF) have long drawn attention as potential biological control agents to manage plant parasitic nematodes. More recently, NF have been studied for their capacity to function as hyperparasites of entomopathogenic nematodes (EPNs) that are employed to manage subterranean insect pests. Given the ubiquity

of NF, surprisingly little is known about their ecology or contribution to the organization of ecological communities. A temporal survey of natural populations of EPNs and five species of NF revealed highly synchronous population growth of *Steinernema diaprepesi* in 2 citrus orchards in central Florida, suggesting an important role of climatic factors. Similar synchrony of population flux at the two sites occurred with the endoparasitic oomycete *Catenaria sp.* and the trapping fungus *Arthrobotrys dactyloides*. We therefore investigated the roles of temperature and competition in the behaviors of these two NF species as they preyed on either the Florida native *S. diaprepesi* or the non-native *S. riobrave*, a species widely used in citrus orchards to manage the *Diaprepes* root weevil. Infective juveniles of *Steinernema diaprepesi* were added to Petri dishes containing *A. dactyloides and Catenaria* sp., either alone or combined, on water agar. Nematode mortality due to *A. dactyloides* was 130% greater ($P<0.001$) in the presence than in the absence of *Catenaria* sp. By contrast, *Catenaria* sp. killed just 38% as many nematodes in the presence of *A. dactyloides,* than in its absence. The combined effects of both NF on EPN survival was always less than additive, suggesting that *Catenaria* sp. efficacy was reduced by an allomone rather than a lack of prey. In the absence of NF, *S. riobrave* survival in sand microcosms was inversely proportional to temperature (15, 18, 21, 24, 27, and 30°C), whereas *S. diaprepesi* numbers were unaffected at temperatures up to 24°C in one experiment and 21°C in a second. The average percentage reduction in nematodes recovered at temperatures above 21°C compared to the lower temperatures was similar for *S. diaprepesi* (34%) and *S. riobrave* (39%). Temperature affected predation by *A. dactyloides* less on *S. diaprepesi* (fungus x temperature interaction, $P=0.13$) than on *S. riobrave* ($P=0.001$). Below 21°C, the average reduction in numbers of *S. diaprepesi* and *S. riobrave* caused by *A. dactyloides* was 31% and 34%, respectively. Above 21°C, however, the average mortality of *S. riobrave* caused by *A. dactyloides* increased to 72% ($P=0.001$), whereas that for *S. diaprepesi* (36%) did not change. Results of these trials and ongoing research on the effects of temperature on EPN population growth in the insect cadaver will be used to develop conceptual models of EPN population biology for comparison with natural patterns observed in the field.

EFFECT OF NITROGEN SUPPLY AND FORM ON THE INVASION OF RICE ROOT BY THE ROOT-KNOT NEMATODE, *MELOIDOGYNE GRAMINICOLA*. **Patil, Jagadeesh[1], A.H. Miller[2] and H.S. Gaur[1*].** [1]Division of Nematology, Indian Agricultural Research Institute, New Delhi-110 012, India. [2]Rothamsted Research, Harpenden, Herts. AL5 2JQ, UK. *Corresponding Author e-mail: hsg_nema@iari.res.in

Rice plant is capable of taking up both nitrate ($NO_3^-$) and ammonical forms of nitrogen (N). It is largely grown under flooded cultivation and $NH_4^+$ is the main form of available soil N. The root knot nematode, *Meloidogyne graminicola* can cause serious damage to rice crops and disturb the N uptake and translocation. We have investigated if the N supply and form can influence the susceptibility of rice to this important pest. Rice plants were cultured in soil supplied with two different N sources at different doses. Roots supplied with a 100-fold lower supply of calcium nitrate (0.1 mM nitrate) showed a higher level of nematode infection as measured by the gall index. A higher root gall index of 4 compared to 2 on a 0-5 scale was recorded for rice supplied with 0.1 and 10 mM $NO_3^-$, respectively. Plants supplied with 2.85 mM of Calcium nitrate were more infected (root gall index = 4.0) compared to the same dose of Ammonium nitrate (gall index = 2) or Ammonium Chloride (gall index = 1). The electrophysiological studies showed that resting cell membrane potentials of nematode infected plants (-53.27 mV) were significantly smaller than those of uninfected control (-70.81 mV) rice. These resting potentials were also not significantly altered by treatment with $NO_3^-$ for both infected (-53.36 mV) and uninfected roots (-70.07 mV). Furthermore, when the cellular responses to $NO_3^-$ were compared, these also showed significantly smaller nitrate transport activity in nematode infected roots (4.7 mV) when compared with uninfected control plants (11.93 mV). Taken together, the $^{15}NO_3^-$ influx and electrophysiological measurements clearly showed that the root $NO_3^-$ transport activity was severely decreased in galled roots infected with nematodes. The results also show that regulation of $NO_3^-$ concentration at critical periods of nematode infection of rice root can provide a non-nematicidal method of nematode management.

AVICTA COMPLETE BEANS – A NOVEL INTEGRATED TOOL TO MANAGE CYST AND NON-CYST NEMATODES IN SOYBEAN. **Pedersen, Palle, C. Watrin, and D. Long.**

Soybean (*Glycines max* L.) nematode damage is difficult to identify and is often misdiagnosed by the grower. Other times, visual symptoms may not be present, yet yield and profit potential are still impacted. It is estimated that a yield loss of up to 40% is possible without any above ground visual symptoms. Nematodes cause severe damage by feeding in or on plant roots, allowing fungi and bacteria to enter and weaken the plant. Root growth is often reduced by nematode feeding, nutrients are removed, and nutrient and water uptake in the roots are disrupted. Many species of nematodes have multiple host plans. Some species, like root-knot nematodes (*Meloidogyne incognita*), are pathogenic on both corn (*Zea mays* L.), cotton (*Gossypium hirsutum* L.), and soybean, that are commonly used in the rotation in the southern United States. Another problem is observed in the north with soybean cyst nematode (SCN) or *Heterodera glycines*, that is the most destructive pathogen of soybean in United

States and cost soybean producers more than $1 billion a year. Several management practices have been investigated to control this pest, such as the use of SCN-resistant cultivars, chemical control (nematicides), and the rotation with nonhost crops. Soybean cyst nematode-resistant cultivars are, by far, the most effective and consistent strategy to control this pest, providing yield advantages in most situations. Unfortunately, most of these cultivars were derived from a few SCN resistance sources, and because of that, virulent SCN populations develop elevated reproduction on cultivars with SCN-resistance. Avicta® Complete Beans nematicide/insecticide/fungicide, a seed-applied combination of Avicta® 500FS seed treatment nematicide and CruiserMaxx® Beans insecticide/fungicide seed treatment, provides soybean growers with protection against damaging nematodes, insect and diseases. This first-of-its-kind seed treatment application is immediate and effective, ensuring soybean seedlings are protected at emergence and early growth stages. With limited soybean nematode control options on the market, Avicta Complete Beans offers protection in the convenience of a seed-delivered treatment – helping to increase plant health and yield potential. Syngenta Seedcare initiated an extensive testing of the yield benefit of Avicta Complete Beans in the southern United States in root knot and soybean cyst nematode infested fields. In one study conducted from 2008 – 2010 across 30 trials, Avicta Complete Beans yielded 3.8 bu/acre more than CruiserMaxx Beans in fields infested with root knot nematodes and with a positive yield response in 76% of the trials. In another study conducted across 28 trials from 2005-2010 in Arkansas and North Carolina, Avicta Complete Beans yielded 3.4 bu/acre more than CruiserMaxx Beans in SCN infested environments with a positive yield response in 72% of the trials. Overall application of Avicta Complete Beans in combination with a moderate resistant cultivar provides reliable protection and allows growers to maximize yield and economic return.

CHARACTERIZATION OF THE GENUS *LABRONEMELLA* (DORYLAIMIDA, QUDSIANEMATIDAE) AFTER SEM STUDY OF A POPULATION FROM IRAN. **Peña-Santiago, Reyes[1], M. Pedram[2], G. Liébanas[1], S. Álvarez-Ortega[1], and J. Abolafia[1*]**. Departamento de Biología Animal, Biología Vegetal y Ecología, University of Jaén, Spain, [2]College of Agriculture, Plant Pathology Department, Tarbiat Modares University, Tehran, Iran.

The genus *Labronemella* is an interesting dorylaimid taxon, member of the subfamily Qudsianematinae in Qudsianematidae, with a dozen species distributed in Europe and Asia. It is mainly characterized and distinguishable from its relatives by having expanded lip region (wider than adjacent body) with oral field visibly sunken (sucker-like) and inner portion of lips differentiated in six very well developed liplets. Other diagnostic features are: odontostyle comparatively slender, guiding ring double, female genital system didelphic-amphidelphic, and tail short and rounded in both sexes. The finding of a population of this genus in Iran allowed studying it under SEM for the first time and obtaining additional information about its morphology, very especially the details of its remarkable lip region. SEM pictures show that lip region is offset by a marked constriction; lips are completely amalgamated, forming a disc- or sucker-like structure, whose anterior surface (oral field) appears distinctly sunken but with its inner (perioral) area differentiated in six large, separate, triangular or trapezoidal liplets protruding on oral field; and inner labial papillae have migrated to the margin of labial disc, being located close to outer labial and cephalic papillae. Besides, cuticle bears fine transverse striation throughout the body, amphid openings are situated at level of labial constriction, vulva is a short transverse slit and ventromedian supplements present the usual arrangement in dorylaims. Lip region pattern is compared to those observed in the genera *Labronema* and *Discolaimus*, belonging to the subfamilies Qudsianematinae and Discolaiminae in Qudsianematidae, respectively. Further data on diversity and taxonomy of *Labronemella* are also provided, including a compendium of its species and a key to their identification.

BIOGEOGRAPHY AND PHYLOGENY OF THE GENUS *CEPHALENCHUS*. **Pereira, Tiago José, M. Mundo-Ocampo and J.G. Baldwin.** Dept. of Nematology, University of California, Riverside, CA 92521, USA.

The genus *Cephalenchus* has been traditionally classified under the family Tylenchidae which mostly includes small tylenchids bearing a short stylet and elongated-filiform tail. Some authors have argued that *Cephalenchus* differs substantially from other Tylenchidae genera and that it therefore should be classified under a separated family. Such arguments have been further supported by molecular evidence based on ribosomal DNA. These phylogenetic analyses show the genus *Cephalenchus* to be more closely related to plant-parasites of higher plants (e.g. Belonolaimidae, Pratylenchidae) and further suggest that Tylenchidae is paraphyletic. Currently, *Cephalenchus* is represented by 20 valid species (based on morphology). The genus has been reported in all continents (except Antarctica) and indeed some species are considered widespread (i.e. *C. hexalineatus* and *C. leptus*). Unfortunately, in extant molecular phylogenies, only one species (*C. hexalineatus*) has been included to represent the genus. This makes difficult testing hypothesis of monophyly of this genus as well as its relationships with other tylenchids. Here, we use representative populations from several sources to revisit this conflicting systematic position using morphological (light and scanning electron microscopy) and molecular (28S and 18S genes) approaches. Seven different populations are included: two from Brazil, (Amazon rainforest and Santa Catarina

state), two from the United States (Yellow Stone National Park, Wyoming and Florida), one from Mexico (Baja California), and two from Vietnam (Cuc Phoung and Nan Cat Tien National Parks). Morphometric data of 21 parameters and qualitative morphological observations suggest that (i) populations from Wyoming and Baja California are very similar (also supported by 28S sequences), (ii) the Florida population fits on the description of *C. illustris* originally described in the Netherlands, (iii) some of the populations included represent species new to science. In addition, our preliminary molecular data when analyzed with other sequences deposited on GenBank show that (i) one of the Vietnam populations is closely related to *C. hexalineatus*, (ii) the population from the Santa Catarina state shows high genetic variability (strongly divergent haplotypes) which suggests the presence of more than a single species occurring sympatrically, (iii) the genus *Cephalenchus* is supported as monophyletic, (iv) the position of the entire *Cephalenchus* clade is supported as outside the Tylenchidae. Finally, we discuss the overall distribution of the genus and its relationship with other tylenchids.

ENHANCING SURVIVAL ATTRIBUTES OF ENTOMOPATHOGENIC NEMATODES. **Perry[1], Roland N., and R.-U. Ehlers[2].** [1]Plant Pathology and Microbiology Department Rothamsted Research Harpenden, Herts. AL5 2JQ, UK and [2]Institute for Phytopathology, Dept. Biotechnology & Biological Control, Christian-Albrechts-University, Hermann-Rodewald-Str. 9, 24118 Kiel, Germany.

Understanding the desiccation survival and heat tolerance attributes of infective dauer juveniles (DJ) of entomopathogenic nematodes (EPN) of the genera *Steinernema* and *Heterorhabditis*, is central to enhancing the shelf-life and field persistence of commercial formulations of these important bioinsecticides. This presentation will review progress in determining the underlying mechanisms for survival of EPN and indicate future developments. Early work on the structural and physiological aspects of desiccation survival focused on the role of the moulted cuticle in controlling the rate of water loss and the importance of energy reserves, particularly neutral lipids. The accumulation of trehalose was also found to enhance desiccation survival and it is evident that this is part of a general response by EPN DJ to increase tolerance of other stresses, including cold, heat and ultra violet radiation. In addition to these structural and physiological aspects, research has expanded on three main lines: to isolate natural populations that can survive harsh environments, such as deserts; to select strains of species, currently in use commercially, which have intrinsic properties that enhance desiccation and heat tolerance; and to utilise molecular data, including ESTs and genome sequence data, to determine the underlying genetic factors that control longevity and stress tolerance of EPN. Tracking gene expression profiles on a whole genome scale will be possible and would identify the nematode's 'desiccome' data, which could be aligned with proteome data. Progress in these areas will enhance our understanding of desiccation tolerance in general, and may specifically result in commercial products with improved longevity and efficacy.

INTEGRATED CONTROL OF ROOT-KNOT NEMATODES IN CALIFORNIA CARROT. **Ploeg[1], Antoon, J.O. Becker[1], and J. Nunez[2].** [1]Department of Nematology, University of California, Riverside, CA 92521, [2]UC Cooperative Extension, Kern County, Bakersfield, CA 93307.

A two-year field trial on a root-knot nematode (*Meloidogyne javanica*) infested site was done to evaluate the effects of biofumigant cover-crops and mustard seed meal on a following fall carrot crop. As a sub-treatment, the carrot seed was either coated with a fungicide mix, or with abamectin in addition to the fungicide mix. Root-knot nematode densities were determined at relevant points in time, and nematode damage and yield of carrot was determined at harvest. Nematode levels increased under the biofumigant cover crops, but were low and not different among treatments at carrot harvest. Mid-season carrot vigor was highest after mustard seed meal and the soil fumigant (Basamid™) control. Sub-plots with abamectin-coated carrot seed also were more vigorous. Carrot yields were highest after Basamid and mustard meal treatments in both years. Averaged over the two years, abamectin seed coating increased yields (kg/m bed) by 50%. Although galling on carrots was minor, the mustard meal and Basamid treatment resulted in the lowest galling in both years. A large percentage of carrots were forked, and therefore unmarketable. The mustard meal and Basamid resulted in the highest percentages marketable carrots. Abamectin seed-coating increased the percentage of marketable carrots in both years by approximately 10%. Combining a pre-plant mustard seed meal treatment with abamectin seed-coating of carrot prevented nematode damage as effectively as Basamid.

LEGACY OF ENTOMOPATHOGENIC NEMATOLOGY: THE EARLY YEARS (1930-1990). **Poinar, Jr., George O.** Department of Zoology, Oregon State University, Corvallis, OR 97331.

Early records and the past history of *Steinernema* and *Heterorhabditis*, their bacterial symbiotes (*Xenorhabdus* and *Photorhabdus*), biology, manipulation, commercialization and utilization are presented. The first entomopathogenic nematode (EPN) to be described was *Steinernema kraussei* from sawfly larvae in Germany by Steiner in 1923. At least 10 additional species of *Steinernema* as well as 3 species of *Heterorhabditis* were described prior to 1990

based on morphological characters of the infective stages and males. The most investigated species during the early years was *S. carpocapsae* described by Weiser in 1955. Glaser was the first to culture an EPN (*S. glaseri*) on solid artificial media in 1931. The associated bacterium of *S. carpocapsae* was first discovered by Dutky in 1937 and described in 1965 by Poinar and Thomas. Further contributions include studies of: the significance of the bacterium in nematode development, the pathogenicity of the bacterium and how it was released inside the insect, the location of bacteria inside the infective stage, the use of evaporative retardants to increase infective stage viability, safety to vertebrates, the desiccation of infective juveniles, methods of mass producing EPNs and efficacy under field conditions. Nematodes were first produced in *Galleria* larvae and crickets, then in containers with solid artificial media and eventually in large fermentation tanks. Miller Corporation was the first company to formulate an EPN product in 1958, followed by Nutrilite Corp. (Biotrol NCS-DD-136) in 1970. The first field release of EPNs occurred in 1932 by Glaser. By 1958, field trials had also been conducted in New Zealand (Hoy), South America (Tang) and Canada (Welch). By 1985, EPNs had been field tested in Australia (Reed & Carne), India (Israel et al.), Russia (Lotin & Ivanova), France (Laumond), Sweden (Burman et al.), Poland, (Sandner & Perowicz), Holland (Simons) and Belgium (Theunissen & Fransen). Application methods ranged from squeeze bottles, watering cans and infested soil to compressed air sprayers.

THE EVOLUTIONARY HISTORY OF NEMATODES. **Poinar, Jr., George O.** Department of Zoology, Oregon State University, Corvallis, OR 97331.

The new field of Paleonematology is launched with a synopsis of all previously known nematode fossils, along with the presentation of new taxa in amber, establishing a total of 49 genera and 91 species of fossil nematodes. Such fossils establish specific lineages at particular times and geographical localities during the earth's history. Fossils from the Precambrian such as *Grypania spiralis* and *Helminthoidichnites meeki* could represent early roundworms. Nematodes from the Paleozoic, Mesozoic and Tertiary Eras are discussed. The earliest known fossil nematode is the Early Devonian *Phytonema phyticum*. *Phytonema*, which lived in the stomatal chambers of the primitive plant *Aglaophyton major*, represents the first herbivore in the terrestrial environment. The first fossil marine nematode is *Nemavermes mackeei* from the Middle Pennsylvanian. The earliest freshwater nematode is the Upper Cretaceous *Captivonema cretacea*. The first free-living terrestrial nematode is the Lower Cretaceous *Vetus libani*. The first insect parasitic nematode is the Lower Cretaceous mermithid, *Cretacimermis libani* in the hemocoel of a chironomid midge. *Ascarites priscus* and *A. gerus* from a Cretaceous dinosaur coprolite represent the earliest known vertebrate parasites. The first known stylet-bearing plant parasite is *Cretaciaphelenchoides burmensis* from the Lower Cretaceous. Of special interest is the first fossil entomopathogenic nematode, a member of the heterorhabditid clade, *Proheterorhabditis burmanicus*. The developmental beetle host still retains some rod-shaped bacteria considered to represent the bacterial symbiont of *Proheterorhabditis*. Representatives of the Acugutturidae, Allantonematidae, Anguinidae, Aphelenchoididae, Ascarididae, Cosmocercidae, Diplogastridae, Filariidae, Heterorhabditidae, Iotonchidae Mermithidae, Sphaerulariidae, Tetradonematidae, Thelastomatidae and Rhabditidae are some of the many new fossils discovered in amber deposits. Many of the nematodes in amber show evidence of ancient patterns of reproductive behavior, disease, phoresis, mutualism and parasitism. Using the principle of behavioral fixity, it is possible to assign many Tertiary nematodes to modern families and sometimes even to extant genera. The longevity and extinction of nematode lineages and the question of nematode population fluctuations at major extinction events in the past are examined. The stem group that gave rise to nematodes is still an enigma. It is highly probable that nematodes evolved in the sea and the first cases of animal parasitism also occurred in the marine habitat. Due to the rarity of nematode fossils, dates for the first appearance of nematode lineages parasitizing plants, invertebrates and vertebrates are estimated from the earliest fossil record of their hosts. The earliest known nematode parasites of humans are also investigated. The oldest report involves eggs of *Ascaris lumbricoides* dated at 30,000 BP (before the present). The four most common nematode parasites of man, *Ascaris lumbricoides*, *Trichurus trichiura, Enterobius vermicularis* and *Ancyclostoma/Necator* have been found in human remains in both the New and Old Worlds.

EFFICACY OF BIO-FUMIGATION AND SOIL SOLARIZATION ON PLANT PARASITIC NEMATODES ASSOCIATED WITH ONION. **Pokharel, Ramesh.**

Soilborne diseases are important constraints for onion production. They do not have effective management options. Efficacy of bio-fumigation is known to manage many soil-borne pathogens of other plant specificity but not in onion especially to nematode pathogen. Studies were conducted to evaluate the efficacy of soil solarization, biofumigation, and their combination on these diseases within the context of existing growers cropping systems. The field studies used a replicated split-split plot completely randomized block design; these were followed by greenhouse experiments. Treatments included mustard vs no mustard as main plots; canola meal cake, chicken manure and control as sub plots; and plastic mulch vs no mulch as sub-sub-plot treatments. Mustard grown until flowering after sweet corn harvest in 2008 was incorporated into the soil along with chicken manure and canola meal

cake in Sept. and temperature sensors were inserted at 15 cm depth to monitor soil temperature. The soil was covered from Sept. 19 to Oct. 30 with four mm thick transparent plastic sheets in designated plots that were made air tight from all sides. In the next season, onion was grown, and towards the end of growing season, the incidence of plant parasitic nematodes was measured. Plastic mulch increased soil temperature, and soil amendments facilitated the process. Onion was grown in the next season, and the incidence of nematode genera was measured toward the end of the growing season. Similar study was conducted in a greenhouse in the next growing season. The study resulted into a high ratio of plant parasites vs beneficial nematodes (20:80) in that onion field that might be an indicating of poor soil health of the field. *Ditylenchus dipsaci, Pratylenchus* and *Meloidogyne* spp. were important nematodes out of nine genera of plant parasitic nematodes (PPN) observed in the present study. Mustard, chicken manure and canola meal cake did not show a negative impact on many PPN populations, however, a reduction in *Pratylenchus* and *Meloidogyne* populations underneath the plastic mulch was observed even when the soil solarization was done in September. No significant effect of bio-fumigation by mustard, canola meal cake, and chicken manure, and soil solarization was observed on different yield components and storage qualities of onion.

TRADITIONAL AND NOVEL METHODS FOR NEMATODE COMMUNITY ANALYSIS. **Porazinska, Dorota L.** Fort Lauderdale Research and Education Center, University of Florida, 3205 College Ave. Fort Lauderdale, FL 33314.

Nematode community analysis depends on diagnostic abilities of component taxa and their abundances. Reliance on morphology and single-organism amplification and sequencing are the core of traditional methodological approaches but come with very well recognized limitations. An ecometagentic approach, driven by the advances in ultrasequencing platforms, allows for a parallel assessment of all individuals in an environmental sample at a cost and time unimaginable by traditional methods. Despite the promise of novel methods opening up new and exciting avenues for research in nematode community ecology (e.g. species resolution, large spatial scales, and biological context), the importance of the progress in the field of traditional methods has never been more clear. The aim of this presentation is to provide a background information on the current state of the ecometagentics and its new directions, but most importantly to identify the gaps in the process requiring input from traditional methods.

A COMPARISON OF TROPICAL AND TEMPERATE RAINFOREST NEMATODE COMMUNITIES USING ECOMETAGENETICS. **Porazinska[1], Dorota L., R.M. Giblin-Davis[1], N. DeCrappeo[2], R.E. Ingham[3], T.H. Nylen[4], and T.O. Powers[5].** [1]Fort Lauderdale Research and Education Center, University of Florida, 3205 College Ave. Fort Lauderdale, FL 33314, [2]USGS Forest and Rangeland Ecosystem Science Center, Corvallis, OR 97331, [3]Dept. of Botany and Plant Pathology 2082 Cordley Hall, Oregon State University, Corvallis, OR 97331, [4]Department of Geology, Portland State University, P.O. Box 751. Portland, OR 97207-0751, [5]Plant Pathology Department, 406 Plant Science Hall, Lincoln, NE 68583-0722.

Longitudinal diversity patterns have been well established for terrestrial macrobiota such as plants and vertebrates, but are often subjects for debates for microscopic organisms such as nematodes. Although a few recent studies have indicated potential support for the hypothesis of increasing diversity from the poles to the equator, the presence of direct evidence is lacking. To help provide this missing data, we conducted a survey of nematode diversity within three habitats (soil, litter, and canopy) at two latitudinally different rainforests in the North American meridian (a tropical rainforest at La Selva Biological Station, Costa Rica and a temperate one at the Olympic National Forest, WA, U.S.A.) using identical sampling designs and sample processing protocols. To access nematode and other microbiotic eukaryota DNA from environmental samples, a parallel ultrasequencing approach was used. The actual and predicted diversity of nematodes across all habitats in a temperate rainforest was ~ 30% that of the tropical rainforest. The distribution of the diversity along specific habitats, however, differed significantly between the rainforests. While soil in the tropical rainforest provided a habitat for only about 20% of all nematode species recovered in the survey, the pattern was completely reversed in the temperate rainforest and consequently the nematode diversity in the soil habitat was significantly higher in the temperate than the tropical region. The composition of nematode taxa across feeding guilds varied between the regions as well with bacterial-feeding species overwhelmingly dominating the tropical rainforest communities and all trophic taxa being more equally distributed in the temperate rainforest. Although more species (~15%) overlapped the three habitats in the temperate rainforest as opposed to the tropical (~2%), the samples maintained their specific habitat signatures at both regions reinforcing the notion of nonrandom spatial species distributions. Overall, we provide for the first time direct evidence for the latitudinal pattern of the nematode diversity. It is necessary to point out, however, that a sampling approach expanding beyond traditionally conceptualized nematode habitats (soil) was crucial in revealing this pattern. It is quite likely that favorable temperature and moisture conditions in tropical regions allow nematode species to exist largely aboveground, whereas in the temperate regions conditions confine nematodes largely to the belowground habitat.

RECOMBINANT DNA IN MULTI-TEMPLATE SAMPLES. **Porazinska[1], Dorota L., R.M. Giblin-Davis[1], W. Sung[2], and W.K. Thomas[2].** [1]Fort Lauderdale Research and Education Center, University of Florida, 3205 College Ave. Fort Lauderdale, FL 33314, [2]Hubbard Center for Genome Studies, University of New Hampshire, 35 Colovos Rd., Durham, NH 03824, USA.

Pyrosequencing of an artificially assembled nematode community of known nematode species at known densities allowed us to characterize the potential extent of chimera problems in multi-template eukaryotic samples. Chimeras were confirmed to be very common, making up to 20% of all high quality pyrosequencing reads and up to 40% of all OCTUs (operationally clustered taxonomic units). Typically, chimeric OCTUs were made up of single or double reads, but double-digit read OCTUs were also present. As expected, the majority of chimeras were formed between two DNA molecules of nematode origin, but a small proportion involved a nematode and a fragment of another eukaryote origin. In addition, examples of a combination of three or even four different template origins were observed. All chimeras were associated with the presence of conservative regions with 80% of all recombinants following a conserved region of about 25bp. Although there was a positive influence of species abundance on the overall number of chimeras, the influence of specific-species identity was less apparent. We also suggest that the problem is not nematode exclusive, but instead applies to other eukaryotes typically accompanying nematodes (e.g. fungi, rotifers, tardigrades). An analysis of real environmental samples revealed the presence of chimeras for all eukaryotic taxa in patterns similar to that observed in nematode mock communities. This information warrants caution for biodiversity studies utilizing a step of PCR amplification of complex DNA samples. When unrecognized, generated abundant recombinant sequences falsely overestimate eukaryotic biodiversity.

A PHYLOGENOMIC APPROACH TO *STEINERNEMA* EVOLUTION. **Camille Porter[1], Dillman, A.R.[2], Mortazavi, A.[2], Sternberg, P.W.[2], Adams, B.J.[1]** [1]Department of Biology, Brigham Young University, Provo, UT 84602. [2]Howard Hughes Medical Institute, Division of Biology, California Institute of Technology, Pasadena, California 91125

*Steinernema* is a genus of entomopathogenic nematodes frequently used for biological control of insect pests. *Steinernema* enters its host insect through natural openings and releases mutualistic bacteria that produce toxins lethal to the insect. In order to better understand the evolution of *Steinernema,* we conducted a phylogenomic study. We estimated the species tree based on the information from the complete genomes of five species of *Steinernema: S. carpocapsae, S. scapterisci, S. monticolum, S. feltiae,* and *S. glaseri.* After doing a homology search, we found approximately 6,000 orthologous single-copy genes common to all five species. We translated the nucleotides into amino acids, aligned them, and then back-translated them to nucleotides for a more biologically meaningful alignment (confident homology statements). We concatenated the alignments of all the genes to facilitate a mixed model Bayesian analysis. We compare our results in the context of previously published analyses based on fewer genes and explore the role of locus choice and taxon selection in phylogenetic analysis.

A PHYLOGENOMIC APPROACH TO *STEINERNEMA* EVOLUTION **Camille Porter[1], Dillman, A.R.[2], Mortazavi, A.[2], Sternberg, P.W.[2], Adams, B.J.[1]** [1]Department of Biology, Brigham Young University, Provo, UT 84602. [2]Howard Hughes Medical Institute, Division of Biology, California Institute of Technology, Pasadena, California 91125

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TAXONOMIC RESOLUTION AND DNA BARCODE ANALYSIS IN ECOLOGICAL STUDIES OF NEMATODES. **Powers, Thomas O.** Department of Plant Pathology, University of Nebraska, 406 Plant Sciences Hall, Lincoln, NE 68583-0722.

DNA barcode analysis is a powerful tool for establishing taxonomic accuracy and consistency in ecological studies. The taxonomic significance of a given barcode sequence depends on a number of factors such as the genetic region under analysis, the rate of evolution of that region, experimental error, and the congruence of gene trees and species trees. Since these factors are seldom completely understood, it is not a trivial question to ask, "What does a barcode represent?" We have taken an empirical approach to answering this question, using different DNA barcodes and different methods of "reading" the barcode, in an examination of species boundaries in

the suborder Criconematina. The genetic regions evaluated include 18S rDNA and ITS1 from the nuclear genome, and cytochrome b and cytochrome oxidase subunit I from the mitochondrial genome. Phylogenetic, distance-based, and character-based methods of barcode analysis have been applied to species identification in traditional approaches to community composition analysis as well as in ecometagenetic approaches.

NUCLEOTIDE CHARACTERS FOR THE IDENTIFICATION OF NEMATODES OF REGULATORY CONCERN. **Powers, Thomas O.** Department of Plant Pathology, University of Nebraska, 406 Plant Sciences Hall, Lincoln, NE 68583-0722.

Molecular diagnostic methods for nematode identification encompass a range of approaches. The drawbacks and limitations of these various approaches are not always apparent. Character-based DNA barcode analysis is a form of analysis that addresses some of the problems inherent in conventional approaches to DNA barcoding. By treating nucleotide substitutions as character states that are coded as present or absent, molecular diagnostics complements traditional morphological identification. Adding discrete molecular diagnostic characters to the descriptions of nematodes of regulatory concern will enhance and accelerate the acceptance of methods of identification based on DNA sequences.

COMPETITION BETWEEN *CAENORHABDITIS BRIGGSAE* NATURAL ISOLATES IN THE PRESENCE AND ABSENCE OF MICROSPORIDIAN INTRACELLULAR PARASITES. **Raboin[1], Michael J., D.K. Howe[2], A.F. Timko[2], M.-A. Félix[3] and D.R. Denver[2].** [1]Molecular and Cellular Biology Program, Oregon State University, Corvallis, OR 97331, [2]Department of Zoology, Oregon State University, Corvallis, OR 97331, and [3]Institut Jacques Monod, Centre National de la Recherche Scientifique, Universities Paris 6 and 7, Paris, France.

Despite their widespread use as models for diverse biological processes, the ecology of nematodes in the genus *Caenorhabditis* is largely uncharacterized. Previous evolutionary and population genetic analyses of *C. briggsae* natural isolates revealed a distinct latitudinal population structure. The ecological causes and consequences of this population structure, however, have not been extensively examined, particularly in terms of inter-isolate interactions and isolate-parasite interactions. To explore the effects of parasitism on *C. briggsae* evolution, we developed a novel assay whereby different isolates of *C. briggsae* compete with one-another in the presence and absence of a microsporidian parasite (*Nematocida* sp. 1) across twenty generations of evolution. Our results suggest that isolates vary in resistance to the microsporidian parasite, and that this resistance incurs a trade-off in fitness in the absence of the parasite. These results may indicate that resistance correlates with phylogeographic structure. Furthermore, some results of the competition approach used here contradict what is expected based on previous measurements of *C. briggsae* isolate-specific fitness measures largely based on fecundity. In conclusion, our study provides a novel experimental approach for understanding relative fitness and evolution in *Caenorhabditis* nematodes; our results demonstrate parasitism-based evolutionary trade-offs in the evolution of this nematode species.

SYSTEMATICS AND DNA CHARACTER-BASED DIAGNOSIS OF THE FALSE ROOT-KNOT NEMATODE *Nacobbus aberrans* (NEMATA:PRATYLENCHIDAE). **Ramirez-Suarez, Angel[1], T. O. Powers[1].** [1]Plant Pathology Department, Institute of Agriculture and Natural Resources, University of Nebraska-Lincoln. 411 Plant Science Hall, Lincoln NE 68583.

The False Root-Knot nematode *Nacobbus aberrans* is distributed from southern Argentina to Ecuador in South America, and in North America it extends from central Mexico to western Nebraska. Throughout its range it causes serious yield losses in a variety of agronomic crops including potato, sugar beet, tomato, bean, spinach, and chard. Since the 1970 synonymization of *Nacobbus batatiformis*, *N. serendepiticus*, and *N. serendepiticus bolivianus* with *N. aberrans* by S. A. Sher, there has been increasing evidence that *N. aberrans* is actually comprised of multiple species. To address this possibility, we have initiated a systematic analysis of the species, integrating morphological, physiological and molecular approaches. Twelve *N. aberrans* isolates were compared representing western Nebraska, two distinct geographic regions of Mexico and the lowlands of eastern Argentina. The physiological host response of the nematodes was tested on tomato and juvenile morphological characteristics were compared by multivariate analysis. Both approaches revealed a slight tendency to differentiate populations. Nucleotide sequencing of the nuclear rDNA ITS1 region and the D2-D3 expansion segments of 28S rDNA gene, together with the mitochondrial region bridging the cytochrome oxidase II gene to the 16S rDNA gene provided strong evidence of molecular differentiation among populations. All three genetic regions were evaluated from individual juvenile specimens that had previously been examined morphologically. Maximum Likelihood and Bayesian Inference of these molecular markers resolved four well- supported groups that displayed a highly structured pattern of geographic distribution. The following discrete groups were recognized: a) Nebraska, b) Mexican north region, c) Mexican central region, and d) the Argentinean lowlands. AMOVA analysis of the four groups demonstrated that the vast majority of the genetic variation was

concentrated among groups rather than within groups. Median-joining spanning network analysis of the mitochondrial marker supported the geographic groupings and estimated that the genetic lineages were separated from each other by 63-92 mutational steps. A character-based barcode analysis of the three molecular markers using the program CAOS (Character Attributes Organization System) identified over 50 diagnostic nucleotide characters for each group. These data strongly support the assertion that *N. aberrans* is comprised of at least four distinct lineages.

EFFECTS OF *MELOIDOGYNE HAPLA* DENSITIES ON GRAPEVINE ESTABLISHMENT. **Riga[1], Ekaterini, J. Wilson[2], J. Pinkerton[3], and I., Zasada[3].** [1]Nematology Division, Northwest Agricultural Products, 821 S. Chestnut, Pasco, WA 99301, [2]Washington State University, IAREC, Prosser, WA, 99350, [3]USDa-ARS, 3420 NW Orchard Av., Corvallis, OR 97330.

The purpose of this project was to evaluate the effect of the root knot nematode, *Meloidogyne hapla*, on vine establishment. Low, medium, and high *M. hapla* population densities were tested on own-rooted Chardonnay and Cabernet Sauvignon varieties for three years. A field site was fumigated with Telone II in the fall 2006 and on the same site, thirty gallon pots were buried and filled with the Telone fumigated soil and each pot was additionally fumigated with Metam Sodium. In spring 2007, soil in the pots was infested with *M. hapla* using low, medium and high nematode densities or left as non-infected controls. Rooted cuttings of Chardonnay or Cabernet Sauvignon were planted immediately after infesting the soil with nematodes. All treatments had 7 replicates per nematode density and grape variety. Nematode population dynamics, and pruning weights were monitored over time while berry weight and trunk weight was collected in the third year. The pot-in- pot experiment was terminated in fall 2010. Soil samples from all pots inoculated with *M. hapla* contained nematodes in all three years except the untreated control soil. The data showed differences amongst the two grape varieties infected with *M. hapla.* Chardonnay grapes were more susceptible to *M. hapla* than Cabernet Sauvignon. Results from this trial will provide grape growers with knowledge that will lead to appropriate management practices and decisions.

*HETERODERA URTICAE*, COOPER 1955, A *HETERODERA GOETTINGIANA* -GROUP CYST NEMATODE FOUND IN ARKANSAS. **Robbins, Robert T[1]., and W. Ye[2].** [1]Department of Plant Pathology, Cralley-Warren Research Center, 2601 N. Young Ave., Fayetteville, AR 72704, [2]North Carolina Department of Agriculture & Consumer Services, Nematode Assay Section, 4300 Reedy Creek Road, Raleigh, NC 27607-6465.

Several cysts were found about the base of a hackberry tree in Toad Suck Park near the Arkansas River in Perry County in October 2010. Only cysts and second stage juveniles were found. The J2 morphometrics agreed closely with those of *Heterodera urticae*, Cooper and so did the cysts though on the small side. *Heterodera urticae* is in Mulvey's group 5 or the H. *goettingiana* –group which also includes the following species: *H. cardiolata, H. carotae, H. cruciferae, H. cyperi, H. goettingiana, H. graminis, H. humuli,* and *H. mothi.* Of these species *H. cyperi* is the only one reported from Arkansas and its J2 are much different morphologically. Most of the J2 morphometics were smaller, but within the range of those of *H. urticae.* In addition the lateral field has four evenly spaced incisures with the outer lines crenate and the outer bands areolated like those of *H. urticae.* The cysts are ambifenestrate like those of *H. urticae.* Molecular analyses of the near-full-length small subunit rDNA gene, D2/D3 expansion segments of the large subunit rDNA gene and internal transcribed spacer revealed this as *H. urticae.* The known distribution of *H. urticae* is the British Isles and Europe. The population is under investigation and a host range as well as more complete morphometrics of J2, females, males, cysts, and other eggs will be determined upon its successful culture.

GENOME SEQUENCING OF THE POTATO CYST NEMATODE *GLOBODERA* ROSTOCHIENSIS. **Rott[1], Michael, M. Belton[1], H. Saeed[1] and G. Smant[2].** [1]Centre for Plant Health, CFIA 8801 East Saanich Rd, Sidney, B.C., V8L 1H3, Canada[2] Laboratory of Nematology, Wageningen Univ., Binnenhaven 5, 6709PD Wageningen, Netherlands.

The potato cyst nematode *Globodera rostochiensis* has gained renewed interest in Canada with the recent discovery of the nematode in Quebec. To better understand the molecular basis for the *G. rostochiensis* pathogenicity and its pathotypes, determination of the *G. rostochiensis* genomic sequence has been initiated using a near isogenic line pathotype Ro1 line, Ro-19. Approximately 10 fold coverage using the Roche 454 GS FLX Titanium system has been completed and additional sequencing using the Illumina GXII system is underway. Some initial analysis will be presented.

NIGHTSHADE HOSTS FOR TWO CANADIAN ISOLATES OF G*LOBODERA ROSTOCHIENSIS.* **Rott, Michael** Centre for Plant Health, CFIA 8801 East Saanich Rd, Sidney, B.C., V8L 1H3, Canada

Two Canadian isolates of the potato cyst nematode (PCN) *Globodera rostochiensis* pathotype Ro1 were evaluated for their ability to replicate on several species of nightshade. Nightshade is commonly found in potato growing fields and can potentially act as an alternative host interfering with eradication/control measures for PCN. Accessions of *Solanum carolinense, S. americanum, S. dulcamera, S. ptychanthum, S.rostratum, S. sarrachoides, S. sisymbriifolium,*

*S physalifolium*, *S triflorum*, *S. villosum* and *S. nigrum*, were evaluated. Of these, most accessions were resistant to PCN with the following exceptions. The Newfoundland (NFLD) isolate of *G. rostochiensis* could replicate on *S. villosum* and *S. dulcamara*. In contrast, the British Columbian (BC) *G. rostochiensis* isolate was found to replicate on *S. dulcamara* but not *S. villosum*. Two of 17 *S. nigrum* accessions were hosts to PCN from NFLD but not BC, however, both of these accessions may not be true *S. nigrum* isolates but *S. nigrum/S. villosum* hybrids.

PHYLOGENETIC RELATIONSHIPS AMONG AGRONOMICALLY IMPORTANT REPRESENTATIVES OF THE ORDER TYLENCHIDA BASED ON MULTIPLE PHYLOGENETIC MARKERS. **Rybarczyk-Mydłowska, Katarzyna D., H.H.B. van Megen, S.J.J. van den Elsen, P.J.W. Mooijman, G. Smant, Bakker, J. and J. Helder.** Laboratory of Nematology, Department of Plant Sciences, Wageningen University, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands.

The nematode order Tylenchida harbors numerous parasites of insect and plants. Gradually, the evolutionary relationships within this economically highly relevant group become clearer. Small subunit ribosomal DNA sequence data suggest that root-knot nematodes (*Meloidogyne*) are nested in and evolved from representatives of the family Pratylenchidae. Moreover, a sister relationship between the families Meloidogynidae and Pratylenchidae on the one hand, and Heteroderidae (cyst nematodes) and Hoplolaimidae on the other was established (Holterman *et al.*, 2009[1]). However, the identity of the member of the Pratylenchidae most close to the common ancestor of all root-knot nematodes remained unresolved. In order to examine those relations more closely, and to better understand the role of horizontal gene transfer (HGT) in plant parasitic nematodes, we employ two additional phylogenetic markers: β-1,4-endoglucanase (pathogenicity related; belonging to glycoside hydrolase family 5) and RNA polymerase II (pathogenicity unrelated). We have successfully developed semi-universal primers for the amplification of the catalytic domain of the cellulase and the largest subunit (RPB1) of RNA polymerase II from single nematodes. So far, our phylogenetic analysis of genomic cellulase sequences (100 sequences from 28 species) confirms the overall topology of the SSU rDNA tree. It additionally provides stronger support for a member of the genus *Pratylenchus* to be the closest to the origin of the root-knot nematode subclade (manuscript in preparation). The basal positioning of *M. artiellia* and *M. ichinochei* within the *Meloidogyne* clade, is confirmed by both cellulase and RPB1 data.

[1]Holterman M, G. Karssen, S. van den Elsen, H. van Megen, J. Bakker, J. Helder (2009) Small Subunit rDNA-Based Phylogeny of the Tylenchida Sheds Light on Relationships Among Some High-Impact Plant-Parasitic Nematodes & the Evolution of Plant Feeding. Phytopathology 99: 227-235.

HOST-PARASITE INTERACTION BETWEEN MERMITHID NEMATODES AND MOSQUITOES. **Sanad[1,2], Manar M., R. Gaugler[1], M.M. Shamseldean[2], Y. Wang[1].** [1]Center for Vector Biology, Rutgers University, New Brunswick, NJ 08901, USA,[2]Dept. of Agricultural Zoology & Nematology, Faculty of Agricultural, Cairo University, El-Gamaa St., Giza 12613 , Egypt.

The mermithid nematodes *Romanomermis iyengari* (Welch) and *Strelkovimermis spiculatus* (Poinar & Camino) are biological control agents attacking a range of mosquito species. We compared various life cycle parameters of these parasites in laboratory exposures against larvae of *Culex pipiens pipiens*. Host search by preparasites (J2) was directed rather than random as indicated in previous studies. Preparasites of both species recognize and prefer to attack uninfected host larvae, a strategy resulting in greater female production. Infection reduces host heart rate and the reduction was correlated with increasing parasitic load. Post-parasite (J3) host emergence location differed sharply, with 93.2% of *R. iyengari* exiting from the anterior prothorax, whereas 100% of *S. spiculatus* emerged perianally. *R. iyengari* exhibits protandry with male post-parasites emerging before females, reflecting the intense male-male competition characteristic of this species; *S. spiculatus* males and females emerge from the host concurrently. Both mermithids displayed the same effect of parasite load on sex ratio, with male production increasing as load increases. As parasite load increased and host resources therefore were depleted more quickly, developmental time in the host decreased. Mating and oviposition occurs within a mating ball or cluster. Females in clusters show accelerated developmental time from post-parasite to the adult stage and mate multiple times. The study of mermithid behavior and factors affecting their life cycle enhances their potential for mass rearing and use in mosquito control programs.

THE ROLE OF TERRESTRIAL MOLLUSKS IN PHORESIS AND VECTORING OF PLANT-PARASITES. **Sanchez[1], Kristi R., S. A. Nadler[1], and E.P. Caswell-Chen[1].** Department of Nematology, University of California Davis, One Shields Avenue, Davis, CA 95616.

Terrestrial mollusks are pests in agriculture, and many of them are significant as invasive pests. The nematode associates of terrestrial snails and slugs are known to include free-living nematodes and parasites of humans and animals. In previous research we observed that the invasive Brown Garden Snail, *Helix aspersa*, often carried

a number of nematode species, including *Caenorhabditis elegans*. We investigated the nematode community associated with *H. aspersa* and observed that *ca.* 90% of the snails we sampled were carrying nematodes. We recovered nematodes from the surface of the snail body and all organs of the snail. The species included plant-parasitic nematodes *Aphelenchoides fragariae, Aphelenchus avenae, Heterodera* spp., and *Xiphinema index*, while bacterial-feeding nematodes such as *Caenorhabditis elegan, Rhabditis terricola,* and *Panagrolaimus* spp. were also found. Bacteria isolated from snail organs and from snail slime included *Escherichia coli*, *Pseudomonas putida*, and *Stenotrophomonas maltophilia*. Also, we have recently isolated fungal pathogens, *Pythium* spp. and *Rhizoctonia* spp. These findings have led us to investigate further and more broadly the role of snails and slugs in the survival, dispersal, and vectoring of nematodes, bacteria, and fungi in agroecosystems because the role of snails and slugs in the dispersal of nematodes and other plant-pathogens is only poorly understood. For example, others have shown that *H. aspersa* may defecate viable propagules (oospores and hyphal fragments) of *Phytophthora citricola*, and snails that feed on *P. citricola* infected avocado can transfer the infection to healthy avocado plants. We are currently investigating the community of plant parasites (bacteria, fungi, and nematodes) associated with snails and slugs in California nurseries, the potential dispersal of nematodes through mollusk phoresis, and the possible role of mollusks in vectoring plant-parasites, including plant-parasitic nematodes. Terrestrial mollusks may disseminate or even enhance invasive pathogens in California agriculture. Given the lifespan of some mollusks and the overall metapopulation structure of snail and slug populations, we propose that the mollusks may be more important than previously recognized in the movement of plant-parasitic nematodes. Terrestrial mollusks are the subject of quarantines, and involvement of mollusks in movement of plant pathogens may require some reconsideration of IPM and regulatory perspectives on those mollusks. Snails and slugs are in intimate contact with soil and plants, and have been investigated as bio-indicators for pollution. These attributes have led us to consider their potential as readily-monitored sentinel or magnifier species to allow detection of invasive pest nematodes, fungi, or bacteria in agroecosystems.

(FLUENSULFONE) MCW-2: A NEW NON-FUMIGANT NEMATICIDE FOR THE CONTROL OF NEMATODES IN CUCURBIT AND FRUITING VEGETABLES (CROP GROUPS 8 & 9). **Schiller, C.T. and J. R. Whitehead.** Makhteshim Agan of North America (MANA), Raleigh North Carolina. 2011.

MCW-2 is a novel broad-spectrum nematicide being developed globally by Makhteshim Agan Industries for the control of nematodes on food crops, turf and ornamental plants. The first Section 3 registration in the USA and Canadian registration for the control of *Meloidogyne incognita* on the Cucurbit and Fruiting Vegetable crop groups is expected in 2013. Early greenhouse and field trials indicate that fluensulfone (MCW-2) at rates of 2 to 4 kg ai/ha (1.78 – 3.56 lb ai/A) applied preplant incorporated significantly reduces the amount of root damage caused by *Meloidogyne* sp in cucurbits and fruiting vegetables. This presentation will focus on the recommended application methods, use rates and timing for control of *Meloidogyne* species in these crops.

NEURONAL PLASTICITY DURING THE DAUER STAGE OF *CAENORHABDITIS ELEGANS*. **Schroeder, Nathan E. and M.M. Barr.** Dept. of Genetics, Rutgers, The State University of New Jersey, 145 Bevier Rd., Piscataway, NJ 08854.

The dauer larva of *C. elegans* is an alternative juvenile stage formed during periods of adverse environmental conditions, and considered homologous to the infective juvenile stage of various parasitic nematodes. Similar to parasitic infective juveniles, *C. elegans* dauers exhibit both altered morphology and behavior compared with non-dauer stages. It is unclear how differences in behavior between dauers and non-dauers are mediated by the nervous system. We discovered that during the dauer stage a set of six inner-labial (IL2) neurons in the head undergo profound and reversible remodeling, as visualized by a transgenic fluorescent reporter. The IL2s are putative chemosensory neurons in the head of *C. elegans*. During non-dauer juvenile and adult stages, the IL2 neurons exhibit a simple bipolar morphology with a single unbranched dendrite extending to the nose and a single axon extending posteriorly. However, in dauers, the two ventral and two dorsal IL2 neurons are multipolar with primary dendrites establishing up to four orders of branching. The two lateral IL2 neurons branch exclusively at the distal dendrites where they form a circular "neuronal crown" extending along the circumference of the cephalic region. Branch formation begins during the 10 hour molt into dauer and consists of a dynamic process of branch formation and retraction. During recovery from dauer (10-14 hours to L4 molt), the branches are partially resorbed, occasionally leaving behind short processes extending from the primary dendrites and cell bodies. We are currently performing a forward genetic screen followed by whole genome sequencing to rapidly clone genes required for proper IL2 branching. We are also using laser ablations to examine cellular interactions which may affect IL2 branching during the dauer stage. Although the function of IL2 branching in dauers is unknown, the morphology is suggestive of a proprioceptive or mechanosensory role. We speculate that IL2 branching may contribute to nictation, a dauer-specific behavior that is also used by the infective stage of several parasitic nematodes. Previous research has shown remarkable conservation of neuroanatomy among phylogenetically diverse nematode

species. We are therefore also examining the structure of putative IL2 homologous neurons in the nictating entomopathogenic nematode *Steinernema carpocapsae* using both dye-filling assays and electron-microscopy.

TEMPERATURE VARIATION IN THE FAME PROFILES OF *MELOIDOGYNE INCOGNITA* AND *M. JAVANICA*. **Sekora[1], Nicholas S. and W. T. Crow[1].** Entomology and Nematology Dept., University of Florida, Gainesville, FL 32611.

Seasonal stresses such as temperature can introduce variation into the physiology of plant-parasitic nematode species, especially fatty acids. Using fatty acid methyl ester (FAME) profiles of *Meloidogyne* species has been proposed as a means for identification in diagnostic laboratories, but the impact of temperature on FAME profiles needs to be evaluated to ascertain the diagnostic applicability of the FAME method throughout the year. Populations of *M. incognita* and *M. javanica* were maintained on *Solanum lycopersicum* cultivar 'Tiny Tim' at 18°C and 28°C. At specified time periods (45, 90, and 135 days) the FAME profiles of roots infected with either species were compared to uninoculated controls to determine what effects, if any, the three temperatures had on their fatty acid content. Both canonical discriminant analysis (CDA) and the Sherlock® Identification Software were used to analyze the fatty acid data. Though statistical differences ($P \leq 0.0392$) were observed in the FAME profiles, initial results indicate that temperature does not inhibit the use of fatty acid-based software to identify *M. incognita* and *M. javanica*. CDA comparisons among temperatures and nematode species were mixed in their significance (ranging from <0.0001 to 0.9974). These differences were primarily due to varying expression of 18:1 ω9t, a fatty acid only found in tomato roots when an infecting nematode species is present. Sherlock® was unable to separate 18°C populations of *M. incognita* or *M. javanica* from 28°C populations of either species but was still able to accurately identify roots infected with *M. incognita* versus roots infected with *M. javanica* regardless of temperature. These results indicate the promising practical applicability of the FAME system for identification of nematode species from plant tissue samples submitted to diagnostic laboratories.

BROAD LABORATORY SCREENING OF ENTOMOPATHOGENIC NEMATODES FOR CONTROL OF PLUM CURCULIO, *CONOTRACHELUS NENUPHAR*. **Shapiro-Ilan[1], David I., T. C. Leskey[2], and S.E. Wright[2].** [1]USDA-ARS, SE Fruit and Tree Nut Research Laboratory, 21 Dunbar Rd., Byron, GA 31008, [2]USDA-ARS, Appalachian Fruit Research Station, 2217 Wiltshire Road, Kearneysville WV, 25430.

The plum curculio, *Conotrachelus nenuphar*, is a major pest of stone and pome fruit in North America. Current control recommendations for *C. nenuphar* consist solely of above-ground applications of chemical insecticides to suppress adults. Due to environmental and regulatory concerns, research on developing alternative control strategies is warranted. Our overall goal is to develop a sustainable multi-stage strategy control *C. nenuphar*. Part of the strategy entails the use of entomopathogenic nematodes (*Steinernema* spp. and *Heterorhabditis* spp.) to control the pest's soil-dwelling stages. In prior research, we established that soil applications of *Steinernema riobrave* can result in high levels of control (78-100%) when targeting *C. nenuphar* larvae in Southeastern peach orchards. Conceivably, when the technology is applied in other regions of the US, e.g., the Northeast, a different nematode may be more appropriate due to varying soil temperatures and other parameters. Therefore, the objective of this study was to conduct a broad laboratory screening experiment to select nematodes for use against *C. nenuphar* in the Northeastern US. Using soil cups containing *C. nenuphar* larvae, we compared the virulence of 13 steinernematid and heterorhabditid strains (comprising eight species) in two different soils (from NH and WV) and at three temperatures (12 °C, 18 °C, and 25 °C). Depending on the soil type and temperature, a number of strains exhibited high levels of virulence including *H. bacteriophora* (Oswego), *H. indica* (HOM1), *S. feltiae* (SN), *S. kraussei*, *S. rarum* (17C&E), and *S. riobrave* (355). Overall, *S. feltiae* (SN), *S. rarum* (17C&E), and *S. riobrave* (355) appear to be the most promising candidates for use against *C. nenuphar* in the Northeast (though *S. riobrave* did not perform as well at the lower temperatures). In future research, we will test the most promising nematodes under various field conditions, and determine their potential for use in an integrated *C. nenuphar* management approach.

APPLICATION TECHNOLOGY FOR ENTOMOPATHOGENIC NEMATODES. **Shapiro-Ilan[1], David I., and C. Dolinski[2].** [1]USDA-ARS, SE Fruit and Tree Nut Research Laboratory, 21 Dunbar Rd., Byron, GA 31008 USA, [2]Universidade Estadual do Norte Fluminense Darcy Ribeiro/CCTA/LEF, Av. Alberto Lamego, 2000, Pq. California, Campos dos Goytacazes, RJ, Brazil, 28015-602.

Diverse technology is available for the application of entomopathogenic nematodes. Application usually consists of nematode distribution via aqueous suspension in various irrigation systems and spray equipment. The choice of application equipment, and method in which the nematodes are applied, can have a substantial impact on pest control efficacy. In addition to application equipment, a variety of other abiotic and biotic factors are of significant concern. The rate of application is critical; in general, a rate of 25 infective juvenile nematodes per $cm^2$ is required for successful pest suppression. Important environmental factors include an adversity to ultraviolet

light, and the need for adequate soil moisture and appropriate temperature. Other agricultural inputs including fertilizers, chemical pesticides and biotic agents (e.g., other biopesticides) can have positive effects on the efficacy of entomopathogenic nematodes, whereas other agents may have neutral or negative effects. In general aboveground applications have been less successful than soil applications due to environmental degradation in the former, but recent research indicates some promise in certain aboveground approaches. Further innovation in application technology will undoubtedly contribute to the expansion of entomopathogenic nematodes as biocontrol agents. For example, novel approaches to application can include distribution of nematodes in their infected hosts, new formulation technology, or prophylactic measures that achieve economic feasibility.

THE STRUCTURE OF INTERTIDAL NEMATODE COMUNITIES ON GULF OF MEXICO BEACHES FOLLOWING THE DEEPWATER HORIZON OIL SPILL. **Sharma[1] Jyotsna**, **H. M. Bik[2]**, **K. Griffith[2]**, **K. M. Halanych[3] and W.K. Thomas[2].** [1]Department of Biology, University of Texas at San Antonio, San Antonio, TX 78249, [2]Hubbard Center for Genome Studies, University of New Hampshire, Durham, NH 03824, [3]Department of Biology, University of Alabama, Auburn AL 36849.

The composition of free-living nematodes communities was studied from 26 intertidal and five subtidal sites in north-east Gulf of Mexico to determine their role in recovery of benthic sediments following contamination by the Deepwater Horizon oil spill. The diversity and composition of the nematode fauna in contaminated sediments is compared to that of available pre-spill sediments and records in the scant literature. Over 3000 individuals from100 species representing 24 families were recovered. The species composition of intertidal sites showed significant differences from the subtidal sites. The species richness of the pre-spill sediments was higher compared to that of post-spill samples. The nematode assemblages at the post-spill beaches were dominated by scavenger and predatory taxa while the pre-spill beaches had a high proportion of deposit feeders. The pre-spill intertidal samples showed high evenness without dominance of a single species while the post spill samples were dominated by the Thoracostomopsidae and Desmodoridae families. The abundance of juveniles suggests the importance of nematodes as r-strategists and early colonizers. The occurrence of nematodes on beaches within a short time after an oil spill suggests that they are important in recovery of contaminated sediments.

A PROTEOMIC DISSECTION OF THE PARASITIC STRATEGY OF THE PINEWOOD NEMATODE. **Shinya[1.2], R., Y. Takeuchi[1], H. Morisaka[1], T. Kikuchi[3], M. Ueda[1], and K. Futai[1].** [1]Graduate School of Agriculture, Kyoto University, Kitashirakawa, Sakyo-ku, Kyoto 606-8502, Japan, [2]Research Fellow of the Japan Society for the Promotion of Science, [3]Forestry and Forest Products Research Institute, 1 Matsunosato, Tsukuba, Ibaraki 305-8687, Japan.

Pine wilt disease caused by the pinewood nematode (PWN), *Bursaphelenchus xylophilus*, has become a "worldwide threat" in recent years. However, little is known about the molecular mechanism of this disease, especially about how PWN attacks the host pine to defeat the host defense responses. In order to reveal the key molecules working at host-parasite interfaces, we focused on the surface coat (SC) proteins and the secretion proteins of PWN, both of which directly contact with host pine cells, and performed a comparative proteome analysis between virulent and avirulent isolates of PWN. The gross amount of PWN SC proteins drastically increased after infection of the host pine. The constituent proteins that characteristically increased after infection, for example reactive oxygen species (ROS) scavengers and detoxifying enzymes, must function in modulating and evading the host defenses. Over one hundred secretion proteins including several ROS scavengers and some cell wall-degrading enzymes were successfully identified by LC-MS/MS analysis. Among them, we detected several intriguing proteins which were secreted significantly greater in virulent isolates than in avirulent isolates. These virulent isolates-specific proteins included proteolytic and lipolytic enzymes. We are currently examining their enzymatic activities and conducting various analyses to figure out their functions. In conclusion, our results suggested that the ROS scavenging proteins accumulated on the body surface of PWN presumably to detoxify ROS generated by the defense responses of host pine, and that proteolytic and lipolytic enzymes secreted from PWNs would potentially serve important roles in their invasion, migration and feeding in the host pine trees. This is the first study to find out the potential virulence factors of the pine wilt disease using comprehensive and systematic approaches. Our data would provide a new insight into the mechanism of pine wilt disease, and into the unexplored parasitic strategy of PWN.

THE PAST AND FUTURE OF BIOLOGICAL CONTROL. **Sikora, R.A.** INRES-Phyto medicine, Soil Ecosystem Phytopathology and Nematology. University of Bonn, Nussallee 9, 53115 Bonn Germany.

The old saying "*The king is dead – long live the king*" could be used to describe the past, present and future of biological control of plant parasitic nematodes. Biological control went through a number of developmental phases that ranged from exploratory over experimental to commercial development. The research in biological

nematode control was both fascinating and frustrating for anyone who entered the arena. The concepts used to research biologicals were targeted at finding something new for publication or toward making a commercial impact. Strategies varied greatly and in many cases development of a marketable biological product was often blocked by too much science and fascination and a lack of knowledge of the industrial marketplace. Complicating the race to make an impact was the looming giant – the nematicides. One learns from history therefore I hope this lecture will allow us to reflect on past successes and mistakes so that the future remains bright for biological management of the worm.

THE WORLDWIDE IMPACT OF SON ON NEMATOLOGY. **Sikora, R.A.** INRES-Phyto medicine, Soil Ecosystem Phytopathology and Nematology. University of Bonn, Nussallee 9, 53115 Bonn Germany.

In 1959 the first name proposed for our society was The Society of North American Nematologists. It was subsequently changed in 1962 to The Society of Nematologists in order to allow nematologists from around the world to be members. After 50 years of existence one wonders – what impact has SON had on nematology on a global scale? SON's opening to the world made an everlasting impact. In our last directory 60 percent of the members were working in North America while an impressive 40 perecent were from overseas. For purely pragmatic reasons, my presentation will focus on the influence North American nematologists had on the discipline globally. Over the past 50 years, SON nematologists working in North America have published extensively and have trained many foreign students in our science. SON has had a major impact on the discipline of nematology worldwide in that many of these students have moved the science of nematology forward in their home countries. SON has helped develop a global-network that promotes progress in the field. Using data gathered from departments, individuals and from past publications an insight will be given into SON's influence on nematology worldwide. The fact that approximately 40 percent of SON members are working outside North America will not go untouched.

COMMERCIAL APPLICATION TECHNOLOGIES AND EFFICACY OF *IN-VITRO* PRODUCED *PASTEURIA* SPP. **Simmons[1], Lee J., T.E. Hewlett[2], J.P. Waters[2], M. C. Doroh[2].** [1]Dept. of Entomology and Plant Pathology, Pesticide Research Building, 411 Research Road, Auburn University, AL 36849; [2]Pasteuria Bioscience, Inc., 12085 Research Dr., Alachua, FL 32615.

*Pasteuria* spp. have been known for decades to parasitize plant parasitic nematodes. The potential use of these bacteria for commercial-scale control of parasitic nematodes has previously been impractical due to the obligate nature of *Pasteuria* and lack of production capabilities. The development of techniques to produce *Pasteuria in-vitro* in fermentation vessels by Pasteuria Bioscience, Inc. (PBI) in Alachua, Florida has now made this possible. Once infective strains of *Pasteuria* have been selected and produced, they require further processing and formulating to become commercially viable agricultural products for nematode control. Commercial formulations must deliver the *Pasteuria* spores to the target zone, aide in efficacy, and be easily adapted to common agricultural practices. Several *in-vitro* produced *Pasteuria* isolates from PBI have been successfully formulated as granular, liquid, and seed treatment products with demonstrated efficacy in turfgrass and agronomic crops reducing plant parasitic nematode populations as well as improving crop quality and yield. Infective *Pasteuria* isolates have been collected from many plant parasitic nematodes and new formulated products for additional crop markets are currently being developed.

EFFECT of VERMICOMPOST-AMENDED SEEDLING-STARTED MIX ON EGGPLANT SEEDLINGS TRANSPLANTED INTO ROOT-KNOT NEMATODE INFESTED SOIL. **Sipes, Brent[1], M. Kermah[2], and T. Radovich[1].** [1]3190 Maile Way, University of Hawaii, Honolulu, USA 96822 and [2]C-SRAD Foundation, P. O. Box 19, Nkroful, Western Region, GHANA.

Agriculture contributes significantly to climate change, therefore developing production systems that reduce greenhouse emissions is a necessary step for climate change mitigation. Vermicompost is one method to reduce greenhouse gas emissions in agriculture. Our objective was to determine the effect of vermicompost-amended seedling-starter mix on the reproduction of *Meloidogyne javanica* and on the growth of eggplant after transplanting. Waimanalo eggplant seeds were sown in a peat-based media amended with vermicompost 0, 25, 50, 75 and 100% (v/v) with no additional fertilizer amendments. Control treatments included 100% organic peat-based mix (OMRI registered peat + perlite) with no supplemental fertilizer and 100% organic peat-based media with Miracle Grow fertilizer (28 g/3.5 l water applied weekly). Six weeks after sowing, the seedlings were transplanted into 10-cm-d clay pots filled with autoclaved soil. Half of the seedlings pots were inoculated with 100 or 500 eggs of *M. javanica* in the first and second runs respectively. Each treatment was replicated 6 times. The plants were harvested 12 weeks later. In both runs of the experiment, nematodes reduced seedling growth regardless of the vermicompost amendment except at the 75% and 100% levels in the first and second runs respectively. In these instances, plant growth was greater in infested soil than in the nematode-free soil. Nematode reproduction was

ten-fold greater in the second run than the first run. In the first run, the 25% amendment resulted highest eggs/plant and the 75% amendment the lowest egg/plant. The highest and lowest were reversed in the second run with the greatest reproduction in the 75% amendment and the lowest in the 25% amendment. The vermicompost-amended seedling-starter mix did not provide a consistent or predictable benefit to the eggplant seedlings.

MOLECULAR AND MORPHOLOGICAL CHARACTERIZATION OF *GLOBODERA* POPULATIONS FROM OREGON AND IDAHO. **Skantar, Andrea M., Z.A. Handoo, L.K. Carta, and D.J. Chitwood.** USDA-ARS, Nematology Laboratory, Beltsville, MD 20705.

An unusual population of cyst nematode was found in soils collected from a Powell Butte, Oregon field with a cropping history including potatoes, wheat, other crops, and significant weed presence. Morphologically, these nematodes possessed characteristics that collectively set them apart from known *Globodera* species. Compared to *G. pallida*, the cyst body length was slightly longer and the second-stage juvenile stylet length was slightly shorter. In some individuals, the J2 stylet knob height was greater and the tail annules were more prominent than in *G. pallida*, and the tail abruptly narrowed with a slight constriction near the posterior third of the hyaline terminus. Compared to *G. rostochiensis,* the hyaline tail terminus had a larger number of refractive bodies, and cysts of this population had a smaller Granek's ratio and fewer cuticular ridges between the anus and vulva. In some individuals, the tail termini of second-stage juveniles were more bluntly pointed, and the stylet knobs were more anteriorly directed with greater height. Unlike *G. tabacum*, the cyst wall often lacked a network-like pattern, and in some individuals, the juvenile tail terminus distinctly narrowed after a constriction. Molecularly, the populations from Oregon and Idaho were similar to each other but distinct from other species in the genus *Globodera*. Multiplex PCR of the ITS rDNA region gave results similar to *G. tabacum*; however, ITS-RFLP patterns were observed to have individual bands in common with *G. rostochiensis* and *G. pallida*. Phylogenetic analysis based on ITS1&2 rDNA sequences showed greatest similarity to populations from Argentina and Chile; together they form a moderately supported clade, distinct from *G. rostochiensis*, *G. tabacum, G. mexicana,* European type *G. pallida*, and several *G. pallida* populations from South America.

AN UPDATE TO THE INTRODUCTION TO NEMATODES: A NEW MULTIMEDIA PRESENTATION. **Skantar[1], Andrea M., E.C. McGawley[2], M.J. Pontif[3], and C. Overstreet[2].** [1]USDA ARS Nematology Laboratory, 10300 Baltimore Ave., Bldg. 011A BARC West, Rm. 165B, Beltsville MD 20705. [2]Louisiana State University Agricultural Center, Dept. of Plant Pathology and Crop Physiology, 302 Life Sciences Bldg., Baton Rouge, LA 70803, [3] Louisiana State University Agricultural Center, Sugarcane Research Station, St. Gabriel, LA 70776.

The Introduction to Nematodes is a multimedia presentation that contains over 100 multi-layered slides comprised of 481 photographs, 155 illustrations, 17 tables and 14 videos. The presentation is formatted as a Quicktime movie and will play on either a Macintosh or a PC computer. The presentation is accompanied by a syllabus, with notes and credits for each slide, an index of the 19 sections (General, History, Morphology, Body Systems, Symptoms, Loss Estimates, Movement & Dissemination, Sampling, Extraction, Population Dynamics, Thresholds, Management, Taxonomy, Parasitism, Key for Identification, Highlighted Genera, Molecular Diagnostics, Disease Complexes and Entomogenous Nematodes), a "read me" file which contains instructions for obtaining and using the Quicktime player, and a set of "thumbnail views" of each slide. This presentation can be obtained as a free download from websites hosted by the Society of Nematologists (SON), the Organization of Nematologists of Tropical America (ONTA), and the European Society of Nematologists (ESN). This presentation will highlight a new section featuring molecular diagnostics of plant-parasitic nematodes.

PERFORMANCE OF ABAMECTIN APPLIED AS A SEED TREATMENT NEMATICIDE. **Slaats[1], Brigitte E., J. Simmons[2], J. Vaz[3], and B. Lovato[3].** [1]Syngenta Crop Protection AG, WST-540.1.07, Schaffhauserstr., 4332 Stein AG, Switzerland, [2]Syngenta Crop Protection LLC, VBRC, 7145 58th Avenue, Vero Beach, FL 32967, USA, [3]Syngenta Protecao de Cultivos Ltda, Av. Nacoes Unidas 18.001, 04795-900 Sao Paulo, Brazil.

Once a field site is infested with plant-parasitic nematodes they are hard to eradicate. Management of nematodes is attainable through traditional crop rotation and cultural measures however such practices are difficult for growers to follow. Due to a growing world population and limited production areas, a demand for higher yields in existing production areas has arisen coupled with increased intensification which is very favourable for nematode multiplication. The use of resistant or tolerant varieties is a common practice to prevent crop loss caused by nematode infestation. However nematode species not targeted by the specific genetic resistance are not affected, multiply and cause yield loss. Due to increasing regulatory pressures there are only a limited number of chemical nematicides still registered with use restrictions. In order for the chemical to be effective the nematicide needs to come into contact with the mobile nematode in the area of the root zone. An effective method of application is as a seed treatment. A seed treatment nematicide can actively protect the young seedling in the first critical weeks of early establishment and secure crop yield from decreases caused by nematode damage. One of the newest classes

of active substances found with nematicidal efficacy is the avermectin class. Avermectins are natural products known for their anthelmintic and insecticidal activities and are also agents which affect parasitic nematodes. Avermectins have limited downward movement in plants when applied as a foliar treatment and need to be applied as granulated or liquid formulations or as a seed treatment to the soil. The first seed treatment nematicide Avicta® was delivered by Syngenta Crop Protection and launched on cotton in 2006. The nematicidal component of Avicta® Complete Cotton is abamectin which is highly active against a broad range of plant-parasitic nematodes and is not just nematostatic but truly nematicidal. During the presentation we will cover the broad spectrum activity of abamectin against a number of plant-parasitic nematode species and the benefit a seed treatment nematicide can add to soybean cyst nematode tolerant soybean varieties.

FOLIAR APPLICATION OF MOVENTO REDUCES REPRODUCTION OF *HETERODERA AVENAE* ON WHEAT ROOTS. **Smiley[1], Richard W., J.M. Marshall[2], and G.P. Yan[1].** [1]Columbia Basin Agric. Res. Center, Oregon State University, Pendleton OR 97801 and [2]Cereals Pathology and Agronomy Program, University of Idaho, Idaho Falls, ID 83402.

The cereal cyst nematode (CCN; *Heterodera avenae*) is present in at least seven western states and reduces yields of small grain cereals in Oregon. Current management requires rotations that include dicotyledonous crops or fallow. Movento is a foliar-applied insecticide (spirotetramat) with ambimobile translocation that reduces fecundity and fertility of sucking insects which feed on roots as well as foliage. Research in California demonstrated that Movento suppressed densities of several nematode species near roots of walnut and grape. During 2010, Movento (5 fl oz/ac) was applied to spring wheat foliage in two CCN-infested fields near Palouse, WA and St. Anthony, ID. The experiment in Washington included a nontreated control and either one or three applications at 2-week intervals. Roots appeared normal when the first application was made but showed abnormal branching at the time of the second application. Swollen white females were visible at the third application. Post-harvest soil extractions included all cysts, including those newly formed and those produced on a previous wheat crop. Compared to the nontreated control, a single or triple application of Movento reduced the post-harvest density of eggs plus juveniles, from cysts, by 78% or 90%, respectively ($P = 0.01$). The experiment in Idaho included a control and either one or two applications. Abnormal root branching and swollen white females were each visible when the first application was made. Compared to the nontreated control, a single or double application of Movento reduced the post-harvest CCN density by 28% or 35%, respectively ($P = 0.03$). When the Idaho experiment was initiated, untreated symptomatic plants were also removed from the field and, with minimal soil adhering to roots, were transplanted into non-infested soil in greenhouse pots. After one-week, 15 plants of equal size were arranged as five replicate blocks with three treatments; control and one or two applications, each at a rate equivalent to 6.25 fl oz/ac. Plants were harvested seven weeks after being transplanted. Cysts from treated plants often contained empty eggs which, for the greenhouse study only, were excluded from counts of 'viable eggs.' Compared to the control, one or two applications reduced numbers of cysts (white plus brown) per pot by 36% or 19% ($P<0.01$), eggs plus juveniles per cyst by 39% or 39% (not significant), and eggs plus juveniles per pot by 78% or 90% ($P = 0.02$). Grain yields and test weights at both field locations were not significantly improved by application of Movento, possibly because juveniles injured roots during initial invasion and intercellular migration, before the females established feeding sites near the phloem and ingested the active ingredient (spirotetramat-enol) in sufficient quantity to reduce fecundity and/or fertility. Movento warrants further evaluation as a substitute for crop rotations or fallow to reduce CCN densities in infested fields.

EXTRACTING CYSTS OF HETERODERA AVENAE USING A TRUDGILL FLUIDIZING COLUMN CONSTRUCTED WITH MODERN MATERIALS. **Smiley, Richard W.** Columbia Basin Agricultural Research Center, Oregon State University, Pendleton OR 97801.

Heteroderid cysts have been extracted from soil using a wide array of equipment and procedures. Construction of automated methods is prohibitively expensive for small laboratories that process relatively few samples. Simple flotation procedures often have low uniformity among repetitions and also produce extracts containing cysts amongst large amounts of organic debris. Separation of cysts from the debris can be accomplished using density-gradient procedures combined with centrifugation but those procedures often reduce the hatching efficiency of eggs that are subsequently released from cysts, making such separations undesirable when eggs plus juveniles are intended to be used as inoculum. Small laboratories need a cyst extraction procedure that is efficient, simple, inexpensive, and neither requires laborious hand picking nor reduces the viability of eggs released from cysts. To extract cysts of *Heterodera avenae*, two versions of the Fenwick can that were constructed for use at our laboratory exhibited a high rate of variability and tended to extract mostly cysts containing few or no eggs. During the 1970s and 1980s, multiple laboratories reported that the Trudgill fluidizing column was more than or as effective as the Fenwick can and Oostenbrink elutriator for extracting cysts of *H. avenae* and *H. schachtii*. The fluidizing column

also tended to extract more egg-bearing cysts than the flask method or a combination of the Fenwick can plus an elutriation step. Moreover, the column produced extracts with little flotation debris, making cysts much easier to count and to collect manually compared to extracts using other methods. Two Trudgill fluidizing columns were constructed using modern materials that were mostly available at a local hardware store. Only three of 26 components were acquired from suppliers via the internet; 'O' rings (Amazon.com), flow-control valves (Freshwater Systems), and custom-molded high-density polyethylene hydrophilic porous plastic fluidizer plate (PolyStar Technologies). The total expense for constructing two columns, the water supply and flow-rate system, and a mounting panel was $253. The 2-column system was constructed in two days using tools commonly available in a home shop. Fluidizing columns at two flow rates were compared with the flask and bucket methods for extracting *H. avenae* cysts from a silt loam, using four replicates of 250 cc subsamples for each method. The column method, at 0.5 and 1.0 gpm, extracted more cysts than the flask or bucket methods; 162, 180, 154 and 150 cysts, respectively (not different at $\alpha$=0.05). The column and flask methods produced cysts clean enough for use as inoculum without further separation from debris. Extracts using the bucket method contained excessive debris. Total time required to prepare, extract and count cysts from individual samples using the column, flask and bucket methods was 22 to 30, 47 to 85, and 60 to 85 minutes, respectively. The Trudgill fluidizing column is useful for small laboratories because it is inexpensive, easily constructed, repeatable, and delivers 'clean' cysts of *H. avenae*.

IMPACTS OF SURFACE COAL MINING RESTORATION ON FREE-LIVING NEMATODE COMMUNITIES. **Smith, Haley.**

Nematodes are considered indicators of disturbance and succession due to their variation in colonization rates and multiple trophic group organization. They play an important aspect of the soil food web by expediting nutrient transfer, which may be beneficial in nitrogen-limited systems. In the eastern United States, surface coal mining has severely disturbed sites in the Appalachian Region. Traditionally, sites have been remediated by compacting soils and planting non-native ground cover. A new method, the Forestry Reclamation Approach (FRA), restores mining sites without soil compaction and utilizes successionally compatible plant species. This study will analyze the effectiveness of traditional reclamation methods and FRA methods by looking at the impact those techniques have on below-ground communities. This study will examine the diversity, abundance, and densities of nematodes in sites that have undergone remediation for 15 years utilizing either the traditional methods or the FRA methods and compare those assemblages with an intact unmined forest. It is expected that the FRA approach will have considerably higher diversity and abundance than sites remediated using the traditional techniques, but not as high as the intact forest.

MOLECULAR PHYLOGENY OF ENOPLIA (NEMATODA): IMPLICATIONS FOR CLASSIFICATION AND STOMA EVOLUTION. **Smythe, Ashleigh.** Dept. of Biology, Hamilton College, 198 College Hill Rd., Clinton, NY 13323.

Members of the subclass Enoplia form one of three primary clades in Nematoda. Enoplia includes Enoplida, primarily aquatic nematodes, and Triplonchida, aquatic and terrestrial nematodes. The presence of presumed ancestral features shared with other animals but no other nematodes have led to the suggestion that Enoplia may represent the earliest nematode lineage. Despite their diversity and evolutionary importance, relatively few members of Enoplia have been included in recent phylum-wide phylogenetic analyses. The taxonomy of Enoplia has also been turbulent in recent years, with several families being transferred from Enoplida to Triplonchida and the placement of certain taxa remaining unclear. This study aims to expand taxonomic sampling of and clarify relationships within Enoplia. Both 18S (SSU) and 28S (LSU) rDNA sequences were determined for members of Enoplia, and phylogenetic trees using maximum likelihood and Bayesian analyses were inferred. Preliminary 18S results indicate that some members of Triplonchida (Tobrilina and Tripylina) are nested within Enoplida. At least one genus in Enoplida, *Viscosia* (Oncholaimidae), appears to be paraphyletic. The phylogenetic framework also allows the opportunity to explore the evolution of morphological features often used in taxonomy, especially feeding structures in the stoma. Preliminary analyses show that fixed teeth and movable mandibles evolved from movable teeth. Clarifying relationships and expanding knowledge of Enoplia will aid in homology assessment of morphological features and determining the root of Nematoda.

HOW *HETERORHABDITIS BACTERIOPHORA* HANDLE THEIR INSECT PATHOGENIC SYMBIONTS. **Somvanshi, V.S.[1], Sloup, R. E.[1], Crawford J.M.[2], Martin, A. R.[1], Heidt, A.J.[1], Clardy J.C.[2], and T.A. Ciche[1].** [1] Dept. of Microbiology and Molecular Genetics, 2215 Biomedical and Physical Sciences Building, Michigan State University, East Lansing, MI 48824. [2] Dept. of Biological Chemistry and Molecular Pharmacology, Rm. C-643 240 Longwood Ave. Harvard Medical School, Boston, MA 02115.

*Heterorhabditis bacteriophora* are entomopathogenic nematodes requiring *Photorhabdus luminescens* bacteria for insect killing and reproduction. Harboring a monoculture of *P. luminescens* in their intestines, infective juvenile

stage nematodes survive, sometimes for many months, before regurgitating the bacteria into insect blood. *H. bacteriophora* are excellent animal models for the study of symbiosis because the symbiosis is simple (monoxenic) and obligate. *H. bacteriophora* is also closely related to the *Caenorhabditis elegans* animal model. We seek to understand how a simple nematode intestine becomes colonized by symbiotic bacteria. We determined that maternal nematodes acquire *P. luminescens* as a persistent biofilm that develops inside the posterior intestine. Only these persistent symbionts are transmitted to infective juvenile nematodes that develop inside of and ultimately consume the maternal body cavity. We determined that the Mad fimbrial adhesive organelle is required for initiating symbiosis. Only a minority of *P. luminescens* cells express Mad fimbriae and initiate symbiosis, while most cells do not. Expression of *mad* is regulated by the stochastic flipping of the invertible promoter *madswitch* to the on orientation. Microscopic observation of *P. luminescens* cells inside maternal nematodes revealed that the persistent biofilm bacteria were smaller (ca. 1/8 vol.) than the larger *P. luminescens* cells which are transiently present. The small cells grew as small colony variants repressed in many characteristics associated with wild type (primary phenotype) such as bioluminescence, antibiotic production and insect virulence. Formation of small colonies was determined to be regulated by the *madswitch*. In summary, the stochastic inversion of the *madswitch* is essential for symbiosis, form and life style switch of *P. luminescens*.

THE STATUS OF NEMATOLOGY TEACHING TO UNDERGRAD AGRONOMY STUDENTS IN BRAZIL. **Souza, Ricardo M. and R.M.G. Silva.** Laboratório de Entomologia e Fitopatologia, Universidade Estadual do Norte Fluminense Darcy Ribeiro, Campos dos Goytacazes (RJ), Brazil. E-mail: ricmsouza@censanet.com.br

Plant nematologists worldwide share the view that growers, technical personnel and other agricultural scientists do not apprehend fully the role of nematodes in the agroecosystems and as plant pathogens. This perceived distortion will likely never be corrected, but it should be minimized. Among other initiatives, plant nematologists and their Societies should strive to improve undergrad Nematology teaching. With this view, a study has been initiated to examine Nematology teaching to undergrad Agronomy students in all Latin America countries. This study has been completed in Brazil, Argentina, Chile, Paraguai and Uruguai. In Brazil there are 151 Agronomy schools, 56.3% public (federal- or state-funded) and 43.7% private. Since some schools have been created recently, Nematology is taught in 139. A questionnaire was sent to these schools, from which 92 professors directly involved with Nematology teaching informed their education background, appointment duties, and area of research; how Nematology is taught and its course load, their evaluation of the appropriateness of the training they offer, the topics in the course syllabus which are poorly taught, and the difficulties faced to improve them. Over 94% of the professors are agronomists. In private schools (Pri Sc), 47% of the professors have a Master degree, and 53% have a Doctorate. In public schools (Pub Sc), this proportion is 7 and 93%, respectively. Although highly educated, most professors have not conducted their dissertation/thesis in Nematology – 88.2% in Pri Sc and 52.6% in Pub Sc. Most professors conduct research activities, although in Pri Sc only 5.6% focus on nematodes only, as opposed to 33.5% in Pub Sc. In 85% of the schools Nematology is taught as a topic within Plant Path courses, with an average course load of 12.8 class-hours (c-h) in Pri Sc and 16.1 c-h in Pub Sc. In comparison with other topics taught in Plant Path, Nematology represents only 0-5% of the c-h in 36.1% of the schools, and about 10, 15 and 20% in 21.7, 15.7 and 13.3% of the schools. Despite this small course load, 48.9% of the professors consider that their students are receiving an appropriate Nematology training. Among the professors that consider that this training is not appropriate, 39% consider the small course load dedicated to Nematology an obstacle to improve teaching. Difficulty to prepare lab classes, difficulty or little time available to update the course contents and poor infrastructure of the schools were also cited. Most importantly, several fundamental Nematology topics are admittedly taught poorly, such as epidemiology and management of nematode-induced diseases, nematode taxonomy and ecology, soil nematode distribution and sampling, and use of entomopathogenic nematodes. An analysis of these and other answers suggest that Nematology teaching of Plant Pathology graduate students must be improved, as many of these students later become Nematology professors who amplify their poor training in fundamental Nematology topics while teaching Agronomy students.

ENTOMOPATHOGENIC NEMATODES AND THEIR BACTERIAL SYMBIONTS: HOW MANY, WHERE AND HOW? **Stock, S. Patricia.** Department of Entomology. University of Arizona. Forbes Bldg. Rm 410. 1140 E. South Campus Dr. Tucson, AZ 85721-0036.

Over the past decades, the field of entomopathogenic nematology has witnessed an exponential growth of new discoveries and research. Initial studies mainly focused on the biological control potential and applications of entomopathogenic nematodes (EPN). We have learned EPN exhibit broad variation in behavior, host range, infectivity, reproduction, and environmental tolerances, among other traits. This biological variation has stimulated interest to more fully characterize EPN's biological, and ecological diversity, towards expanding the usefulness of these organisms as biological control agents against agriculturally important pests. In this respect,

a worldwide search for better adapted species and isolates has grown in recent years. This explosive growth of newly discovered taxa has promoted and demanded the search for new and improved taxonomic tools for species identification and diagnostics. Moreover, recent findings indicate that both *Steinernema* and *Heterorhabditis* have evolved both specialized and generalized relationships with their bacterial symbionts, with varying degrees of dependence. The inherent diversity of this system has also propelled research in the fields of ecology, evolution, biochemistry, and molecular genetics. Moreover, the experimental tractability of this EPN-bacterium mutualism makes it valuable model system to study physiological, chemical, structural and developmental aspects of beneficial symbiotic associations. In this presentation, I will review the current state of the taxonomy, phylogenetic relationships and biological diversity of these nematodes and their bacterial symbionts.

USE OF *HETERORHABDITIS BACTERIOPHORA* & *STEINERNEMA RIOBRAVIS* IN BIOLOGICAL CONTROL OF THE LARGER BLACK FLOUR BEETLE AND THE ROLES OF SOIL TYPE AND SOIL MOISTURE CONTENT. **Stokes, Bryan C.[1], C. Nansen[1,2], and T. A. Wheeler[2].** [1]Department of Plant and Soil Sciences, Texas Tech University, [2]Texas AgriLife Research and Extension Center Lubbock.

The larger black flour beetle (*Cynaeus angustus,* LBFB) can thrive in cotton gin trash piles in the Southern High plains of Texas, and it is considered an occasional nuisance pest to nearby homes and businesses. Adults and larvae feed on saprophytic fungi and have been observed to burrow into the soil underneath piles. The use of entomopathogenic nematodes as biological control agents is being researched as part of identifying feasible tactics to reduce LBFB populations in gin trash piles. A Laboratory experiment was conducted using infective juveniles of two entomopathogenic nematodes (*Heterorhabditis bacteriophora* and *Steinernema riobravis*) in soils containing five different clay contents (63, 48.4, 33.8, 19.1, and 4.5%) at a 15% soil moisture content by weight. Persistence and virulence of both nematodes were observed over a seven day period. The persistence of both nematode species was negatively correlated with time and soil clay content. The virulence of *H. bacteriophora* on LBFB larvae was negatively affected by clay content but was unaffected by time. The virulence of *S. riobravis* was not affected by clay content, but showed a decrease in causing mortality to LBFB larvae overtime. The second laboratory experiment consisted of examining persistence and virulence of *H. bacteriophora* and *S. riobravis* over a 28 day period in one soil type with five different soil moisture contents (5, 10, 15, 20, and 25% by weight). The persistence of both nematodes was highest at the 15% soil moisture content and was negatively correlated with time.

DEFENSIN-LIKE ANTIBACTERIAL FACTORS (ABFS) IN NEMATODES. **Tarr, D. Ellen K.** Department of Microbiology and Immunology, Arizona College of Osteopathic Medicine, Midwestern University, Glendale, AZ 85308.

Nematode defensin-like molecules were first isolated from *Ascaris suum* (ASABFs=*A. suum* antibacterial factors), and later from *Caenorhabditis elegans* (Ce-ABFs). Expression patterns and *in vitro* antimicrobial activity have been consistent with a role in defense against pathogens, and their presence in two distantly related species suggests this role is not species specific. However, these effectors have been notably absent from *Brugia malayi* and *Meloidogyne incognita*, complicating the argument for their importance in nematode immunity. Previous analyses have proposed a common ancestor for nematode ABFs and mollusk defensins, as well as the alternative hypothesis that mollusk and arthropod defensins have a common ancestor with similarity to nematode ABFs the result of convergence. While these previous analyses included only the sequences from *A. suum* and *C. elegans*, a search of the databases identified ABFs in a total of 25 species from clades I, III, IV, and V (nematodes from clade II are not currently well represented in the databases). Information is incomplete for most nematode species, but the number and diversity of taxa suggest these effectors are part of nematode innate immunity that has been lost in some nematode lineages, rather than gained in a select few. Surprisingly, *M. hapla* has ABFs, even though *M. incognita* does not. Bayesian analysis of this dataset did not support either of the previous models for the evolution of invertebrate defensins, with some nematode ABFs showing greater homology to arthropod defensins. There has been little direct work characterizing this group of effectors *in vivo*, but *Ce-abf5 -/-* worms show increased mortality when fed on plates seeded with *Staphylococcus aureus*. Although this is consistent with a role in defense, bacteria are a food source for *C. elegans* as well as potential pathogens. A digestive role has not yet been proposed for ABFs (as it has been for caenopores and lysozymes), but further studies are needed to differentiate between a defensive and digestive role for these effectors.

BACTERIAL CYANOGENESIS AND ITS ROLE IN BIOCONTROL OF PLANT-PARASITIC NEMATODES. **Taylor[1], Christopher G. and P.A. Okubara[2].** [1]Department of Plant Pathology, Ohio State University, Ohio Agricultural and Research Development Center, 210 Selby Hall, 1680 Madison Ave., Wooster, OH 44691 and [2]USDA-ARS, Root Disease and Biological Control Research Unit, Washington State University, 367A Johnson Hall, Pullman, Washington USA 99164.

Plant-parasitic nematodes are among the most destructive plant pests, causing substantial economic losses to agronomic crops worldwide. Current methods of using bacteria as biocontrol agents for plant-parasitic nematodes have met with limited success in part due to limited knowledge about mechanisms of biocontrol and biotic factors that are important to rhizosphere persistence. Using a *C. elegans* bioassay we have screened over 10,000 bacterial isolates from a variety of natural sources (water, soil, plants) and identified over 50 different isolates of *Pseudomonas* that interfere with nematode growth and development. Over a quarter of these strains exhibit activity in plate and soil assays against root-knot and soybean cyst nematodes. We have characterized the nematode-active *Pseudomonas* isolates for motility, exoprotease activity and production of siderophores, hydrogen cyanide (HCN), polysaccharides, and fluorescence to determine if commonalities exist among plant-parasitic nematode lethal strains. Using a transposon knockout strategy, we identified several *C. elegans* non-lethal isolates for *Pseudomonas* strain 15G2. Testing of the non-lethal transposon tagged isolates for HCN showed significant reduction in HCN production. Targeted mutations of the HCN locus also resulted in reduction in HCN production in plates and in the rhizosphere. Loss of HCN production was correlated with loss of activity against *C. elegans* in bioassay plates and reduced capacity to protect plants from plant-parasitic nematodes in pot assays. These data indicate that HCN is potentially an important compound produced by pseudomonads within the rhizosphere with activity against plant-parasitic nematodes.

COMPARITIVE GENE EXPRESSION ANALYSIS OF MAIZE TRANSFER CELLS AND ROOT-KNOT NEMATODE INDUCED GIANT CELLS TO ANSWER THE QUESTION, "DO GIANT CELLS SHARE A COMMON FUNCTION WITH OTHER TRANSFER CELLS?". **Taylor[1], Christopher G., L.M. McIntyre[2], K.A. Koch[3].** [1]Department of Plant Pathology, Ohio State University, Ohio Agricultural and Research Development Center, Wooster, OH 44691, [2]Molecular Genetics and Microbiology Program, University of Florida, Gainesville, FL 32605, and [3]Horticultural Sciences Department, University of Florida, Gainesville, FL 32605.

Plant systems adapt to the mass flow of nutrients across apoplastic spaces through the development of specialized cells called transfer cells. These cells are found in all taxonomic plant groups and are formed during organ development. In maize seed development, transfer cells form in the developing endosperm adjacent to maternal vascular tissues of the cob. In plant pathology, root-knot nematodes (RKN) induce the formation of specialized feeding site consisting of several cells called "giant cells" (GC) with transfer cell-like characteristics. Due to their functional similarities, transfer cells from seeds and root-knot nematode feeding sites share many of the same morphological characteristics including thickened and highly invaginated cell walls, dense cytoplasm, abundant ER, and numerous small vacuoles and mitochondria. These similarities have motivated us to use a comparative transcriptomic approach towards the identification of genes that are expressed in these cells and determine their function. Using laser microdissection technology we have isolated RNA from a time-course series of transfer cells from developing seeds of maize (basal endosperm transfer layer) as well as GC of maize nematode-infested roots. Comparative analysis of microarrays will be used to identify genes (if any) that may be shared between these two cell types.

SOIL NEMATODE COMMUNITY RESPONSE TO CROPPING MANAGEMENT AND CONVERSION TO NATIVE PRAIRIE GRASSES IN A COLD CONTINENTAL CLIMATE. **Tenuta[1], M., S.S. Briar[1], C. Barker[2], and M. Entz[3].** [1]Department of Soil Science, [2]Formerly Department of Soil Science, and [3]Department of Plant Science; University of Manitoba, Winnipeg, Manitoba, Canada R3T 2N2.

Soil nematode community response to treatments of three, four-year crop rotations (Spring Wheat-Pea-Spring Wheat-Flax, Spring Wheat-Green manure-Spring Wheat-Flax, and Spring Wheat-Alfalfa-Alfalfa-Flax) under Conventional and Organic management, and native tall grass restoration (Restored Prairie) were assessed in June 2003, and July and August 2004. The research site was the Glenlea Long-term Rotation and Crop Management Study, in the Red River Valley, established in 1992. The nematode community varied most with sample occasion than management and rotation. The Restored Prairie favored high c-p (colonizer-persister) value omnivores and carnivores, and fungivores but less bacterivores. The Restored soil food web was highly structured, mature and low-to-moderately enriched as indicated by structure (SI), maturity (MI) and enrichment (EI) index values, respectively. Higher abundance of fungivores and channel index (CI) values suggested fungal-dominated decomposition. Nematode diversity in the Restored Prairie was low even after more than a decade of restoration. A longer time may be required to attain higher diversity for this restored fragmented prairie site distant from other native prairies locations. No consistent differences were found between Organic and Conventional management for nematode trophic abundance except enrichment opportunists of the c-p 1 group were favored by Conventional management. Although EI was lower and SI was higher for Organic than Conventional management their absolute values suggested decomposition channels to be primarily bacterial and relatively fewer trophic links with both management regimes. A high abundance of fungivores in the rotation including the green manure crop

indicates greater fungal decomposition. Study results indicate organic systems may attain better structured soil food webs if mechanical disturbance is minimized coupled with no chemical inputs.

PRELIMINARY REPORT OF THE ROOT KNOT NEMATODE, MELOIDOGYNE INCOGNITA, DAMAGING PACHYRHIZUS SP IN PERU. **Tenuta[1], Mario., T.Z. Felde[2], W. Gruneburg[2], O.I. Molina[1] and S.S. Briar[1].** [1]Department of Soil Science, University of Manitoba, Winnipeg, Manitoba, Canada R3T 2N2 and [2]International Potato Centre, La Molina, Lima, Peru.

*Pachyrhizus* is a genus of tropical/subtropical legumes having edible taproots. Three species are cultivated, *P. erosus* (yam bean) *P. tuberosus* (Amazonian yam bean) and *P. ahipa* (Andean yam bean). The International Potato Centre (CIP) in Peru has research program to increase the nutritional profile of this crop. However, field studies for growing breeding crosses at several locations has been hindered by plant losses due to diseases. A total of 70 tuber samples collected from field site planted with accessions of the three species of *Pachyrhizus* and crosses at the CIP in Peru, were analyzed for the plant-parasitic nematodes, and damage (galling index) on tubers. Important morphological characters of males, juveniles and female perineal patterns were consistent with that of *Meloidogyne incognita*. Female perineal patterns were oval to round and the lateral field was weakly demarcated by forked striae. Amplification of the non-transcribed spacer region between the 5S and 18S rDNA genes with the 194/195 primers yielded 720 bp product. MI-F/MI-R specific SCAR primers produced 999 bp fragment and was consistent for *M. incognita* identification. 95% of the tubers had galls while all tubers were infested with nematode. 55% of the tubers had 40% or more galling on them. Galls on the tubers were spherical and/or ellipsoidal in shape. Although there have been observations previously of galling due to RKN on *Pachyrhizus* sp, to our knowledge this is the first study to demonstrate severe damage by *M. incognita*. The results continue to be analyzed for accessions suitable and tolerant of *M. incognita*.

RESISTANT ROOTSTOCKS FOR MANAGING ROOT-KNOT NEMATODES IN GRAFTED WATERMELON. **Thies, Judy A.[1], R. Hassell[2], J. Ariss[1], and A. Levi[1].** [1]U.S. Vegetable Laboratory, USDA, ARS, Charleston, SC 29414 and [2]Clemson University, Coastal Research and Education Center, Charleston, SC 29414.

Southern root-knot nematode (*Meloidogyne incognita*) significantly reduces watermelon yields in the southern U.S. Pre-plant fumigation of soil beds with methyl bromide has been the primary method for controlling root-knot nematodes (RKN) in watermelon. However, the reduced availability and escalating cost of methyl bromide for pre-plant soil fumigation has stimulated interest in the development of new methods, such as grafting on resistant rootstocks, for managing RKN in watermelon. A seedless watermelon scion ('Tri-X 313') was grafted on six different rootstocks and evaluated with and without methyl bromide fumigation in a field infested with RKN at the U.S. Vegetable Laboratory, Charleston, S.C. in 2010. Three wild watermelon lines developed by our team (*Citrullus lanatus* var. *citroides* RKVL 301, RKVL 308, and RKVL 316), 'Emphasis' bottle gourd (*Lagenaria siceraria*), 'Strong Tosa' squash (*Cucurbita moschata* x *C. maxima*) hybrid, 'Ojakkyo' wild watermelon rootstock (*C. lanatus* var. *citroides*), and non-grafted 'Tri-X 313' were included in the study. The RKVL wild watermelon rootstocks exhibited resistance or tolerance to RKN with percentages of root system galled ranging from 11% for RKVL 316 to 56% for RKVL 301 in the untreated control plots. 'Ojakkyo' watermelon, 'Emphasis' bottle gourd, and 'Strong Tosa' hybrid squash rootstocks, and non-grafted 'Tri-X 313' seedless watermelon had 52%, 97%, 96%, and 96% galling, respectively. Fruit yields in the untreated plots were 21.9, 25.6, and 19.9 kg/plot for RKVL 301, RKVL 316, and RKVL 318, respectively. Yields were significantly greater ($P<0.05$) for the three RKVL rootstocks than for 'Strong Tosa' (3.0 kg), 'Emphasis' (7.2 kg), and 'Ojakkyo' (2.8 kg) in the untreated plots. Methyl bromide was highly effective in controlling RKN in all rootstock entries with essentially no galling observed for any entry. Yields of watermelon grafted on 'Strong Tosa' were nearly 7X greater ($P<0.05$) in the methyl bromide treated plots than in the untreated plots. In contrast, yields of RKVL 301, RKVL 316, and RKVL 318 were similar in both treatments. The bottle gourd and hybrid squash rootstocks were highly susceptible to RKN with severe root galling and very low yields in untreated plots, demonstrating that bottle gourd and hybrid squash are unsuitable for use in RKN-infested fields without methyl bromide or other nematicide treatment. The three RKVL wild watermelon rootstock lines exhibited resistance/tolerance to RKN. RKVL 316 had low root galling and produced the heaviest fruit yield and greatest numbers of fruit of any rootstock evaluated. The RKVL lines should be useful sources of RKN-resistance for rootstocks for grafted watermelon. Also, the RKVL lines should be useful in the development of RKN-resistant watermelon cultivars.

*MELOIDOGYNE PARTITYLA*-INDUCED CHANGES IN PECAN GROWTH AND NUTRIENT SEQUESTRATION. **Thomas[1], Stephen H., J.M. Trojan[1], and R.J. Heerema[2].** [1]Dept. of Entomology, Plant Pathology and Weed Science, N141 Skeen Hall, and [2]Extenstion Plant Sciences Dept., N140 Skeen Hall, New Mexico State University, Las Cruces, NM 88003.

Pecan root-knot nematode (*Meloidogyne partityla*) was first reported in the USA in 1996 from orchards in several counties in Texas. Since then the parasite has been recovered from declining pecan trees expressing canopy

dieback and/or mouse-ear leaf symptoms in Arizona, Florida, Georgia, Oklahoma, and New Mexico. In 2005 a study was initiated to determine the effect of the nematode on performance of 'Burkett' and 'Riverside' pecan rootstocks which are commonly found in mature orchards in the Southwest. In May 2005, two year old greenhouse-grown seedlings were planted in 0.85m-diam. field microplots containing 300 liters of soil that was either infested with *M. partityla* or non-infested with this nematode (control). The study was designed as a 2 cultivar x 2 inoculum level factorial with all treatments replicated five or more times, and was terminated in 2008. During the 2006, 2007 and 2008 growing seasons trees were irrigated and fertilized according to regional recommendations, and leaf nutrient levels, plant moisture stress and growth parameters were measured. At the conclusion of the study, all trees were destructively sampled. Roots were separated by size, and those less than 3 mm in diameter were bulked, chopped, and *M. partityla* eggs were recovered and quantified from a 25 g subsample of chopped roots from each tree. The remaining roots from each tree, along with leaves and shoot wood, were dried and weighed. There were no statistically significant differences between rootstocks with respect to final *M. partityla* reproduction rates per gram root tissue. Few differences in leaf nutrient levels or plant growth parameters were detected between infected and non-infected trees during the first two years of the study. However, trees infected with *M. partityla* showed significant reductions in leaf biomass (-53%; $P$=0.088) and trunk diameter (-31%; $P$=0.081) at termination of the study. Conversely, the concentration of total Kjeldahl nitrogen was higher ($P$=0.080) in roots of infected trees (1.281 ± 0.092%) compared to non-infected trees (1.043 ± 0.093%) at the end of the study. Stem water potential was more negative ($P$=0.098) in infected trees (-0.816 ± 0.036 Mpa) than non-infected trees (-0.727 ± 0.036 Mpa) – an indication of greater moisture stress where nematodes were present. As previously reported (Nyczepir at al., 2006, HortScience 41:402-404), 2008 foliar Ni concentrations were lower in infected trees (-28%; $P$=0.046), whereas other foliar nutrient concentrations were unaffected by *M. partityla*. These results indicate that pecan root-knot nematode affects young trees under pseudo-field conditions in the irrigated, high pH soils of the Southwest by reducing above-ground growth and inducing Ni deficiency, moisture stress and nitrogen accumulation in roots. Further research is needed to ascertain potential varietal differences in susceptibility to or tolerance of pecan to *M. partityla*.

LIFE CYCLE OF *PLECTUS MURRAYI* UNDER LABORATORY CONDITIONS. **Cecilia Milano de Tomasel,[1] B.J.Adams,[2] B.NAdhikari,[3]and D.H. Wall[1]** [1]Department of Biology and Natural Resource Ecology Laboratory, Colorado State University Fort Collins CO 80523 [2]Department of Biology and Evolutionary Ecology Laboratories, Brigham Young University Provo, UT 84602 [3] Department of Plant Biology, Michigan State University, East Lansing, MI 48824.

The bacterial feeder *Plectus murrayi* is a species endemic to continental Antarctica and is one of four nematode species found in the McMurdo Dry Valleys region, considered one of the most extreme desert ecosystems on Earth. Nematodes constitute the dominant invertebrate in soil food webs and play important roles in carbon cycling and nitrogen mineralization. Our goal was to study the *P. murrayi* life cycle under laboratory conditions. Soils from the Dry Valleys were shipped to Colorado State University, where nematodes of all stages were extracted and gravid females were cultured in a 15°C incubator in the dark, on a culture medium of Bold's Modified Basal Freshwater Nutrient Media and Ottawa sand (granular silicon dioxide). Higher temperature was selected based on results from Overhoff with *Scottnema lindsayae* life cycle from the Dry Valleys. Gravid females were observed daily until eggs were laid and hatched. It was observed that the first and second juvenile stages occur inside the egg. The second stage juveniles hatched an average 10 days (range 7-14) after eggs were laid, with an average hatching length of 400 ± 50 μm. Sixty-four juvenile individuals were hand picked right after hatching, each one moved to separate plates, and followed daily until they died or became adults. Right after hatching the second stage juveniles underwent a second molting, reaching the third stage of development 7-10 days after emerging from the eggs. The nematodes reached the fourth stage 17-20 days after hatching, becoming adults 30-32 days after hatching. Adult individuals had an average length of 950 μm, with range of 850μm to 1050 μm. These adult nematodes laid eggs 41-43 days after hatching, resulting in a total life cycle length (from egg to egg) of 48-57 days for *Plectus murrayi*.

THE EFFECT OF PLANT-DERIVED PRODUCTS ON THE MOTILITY AND SURVIVAL OF *MELOIDOGYNE JAVANICA*. **Venter, Chante, H. Fourie*, and L. Tiedt.** School of Environmental Sciences and Development, Plant Protection, Private Bag X6001, North West University, Potchefstroom, 2520, South Africa.

Due to the progressive withdrawal of Class 1 synthetic nematicides from local and international markets, a demand exists for environmentally-friendlier products that exhibit nematicidal/nematostatic properties. Although a wide range of plant-derived products have been studied extensively to determine their effect on the biology and survival of plant-parasitic nematodes, newly developed ones are continuously being released. The effect of three such test products at a dosage rate of 500ppm was initially evaluated *in vitro* (at 26°C) 24, 48, 72 and 96 hours after onset of the trial on the motility and survival of second-stage juveniles (J2) of *M. javanica*. Between 50 and 60 actively moving J2 were handpicked and suspended in 2ml of these products. Also included were three commercially-available products

(standard treatments), salicylic acid and an untreated control consisting of tap water. Separate follow-up trials with each of the three test products included a range of dosage rates (400, 800, 1600, 3200 and 6400ppm) and the same intervals mentioned for the 500-ppm trial. All four trials consisted of randomised complete block designs with four replicates for each treatment. For the 500-ppm trial, the untreated control treatment resulted in 92% to 97% of J2's being motile at all of the respective time intervals. Data for J2's suspended in the three commercial standards, salicylic acid and the three test products, however, varied substantially in term of their motility for the duration of the trial. For the three dosage-response trials, significantly higher numbers of J2, compared to the untreated control, were immotile for Test Product 1 from the 800-ppm dosage rate from 48 hours onwards. Although the same effect was evident for Test Products 2 and 3, the majority of J2's was again motile after 72 and 96 hours. Salicylic acid was the only product with a 100% mortality rate for J2's, for both the 500ppm as well as the dosage-response trials, when stained with Tripan Blue after 96 hours. The effect of all treatments included in this study on the ultrastructure of J2's is currently being conducted by means of electronmicroscopy.

DNA BARCODE-BASED TOOL FOR THE MONITORING OF NEMATODE ASSEMBLAGES. **Vervoort[1], Mariëtte T.W., P.J.W. Mooijman[1], H.H.B. van Megen[1], S.J.J. van den Elsen[1], K.D. Rybarczyk[1], J.A. Vonk[2], C. Mulder[2], P.C. De Ruiter[3], J. Bakker[1] and J. Helder[1].** [1]Laboratory of Nematology, Wageningen University, Droevendaalsesteeg 1, 6708 PB, Wageningen, The Netherlands. [2]Laboratory for Ecological Risk Assessment, National Institute for Public Health and Environment (RIVM), A. van Leeuwenhoeklaan 9, P.O. Box 1, 3720 BA Bilthoven, The Netherlands.[3]Centrum Bodem, Wageningen University, Droevendaalsesteeg 4, 6708 PB, Wageningen, The Netherlands.

Within the vast complexity of the soil food web, nematodes are an informative group due to their representation at three different trophic levels. Environmental stress is not only reflected if it affects nematodes directly, but also if it results in major changes in (for instance) bacterial or fungal communities. Although nematodes possess several additional assets preferred for a bio-indicator (*e.g.* easy extractability, differential sensitivities to disturbances, ecological interpretability), the microscopic identification of so-called mass-slides demands skills, lots of time and endurance. For this reason, we have developed a database of currently ≈ 2,300 full-length small subunit ribosomal DNA sequences (appr. 1,700 bp each) from representatives of most major terrestrial and freshwater taxa (*e.g.* Van Megen *et al.* 2009). ARB (Linux-based freeware developed within the microbiology community) was used to design family and genus-specific PCR primers (all with an annealing temperature of 63°C). Currently, about 40 quantitative PCR assays are available for the analysis of nematode communities at family, genus or species level. Results will be presented of a field experiment in which 16 nematode families and genera were sampled every other week during a full season (period of 10 months). The results demonstrate that important ecological information is lost if nematodes are lumped into feeding groups. Substantial seasonal differences were observed in presence and densities of individual families and genera belonging to the same trophic group.

Van Megen H, Van den Elsen S, Holterman M, Karssen G, Mooyman P, et al. (2009) A phylogenetic tree of nematodes based on about 1200 full-length small subunit ribosomal DNA sequences. Nematology 11: 927-950.

CELL CYCLE MANOEUVRING: A STRATEGY TAKEN BY PLANT PARASITIC NEMATODES TO INDUCE SPECIALIZED FEEDING SITES IN PLANT ROOTS. **Vieira, Paulo[1,2,3,4], G. Engler[1,2,3], M. Mota[4], P. Abad[1,2,3], L. Veylder[5], and J. Almeida-Engler[1,2,3].** 1INRA, UMR, 1301, 400 route des Chappes, F-06903 Sophia Antipolis, France, 2CNRS, UMR 6243, 400 route des Chappes, F-06903 Sophia Antipolis, France, 3Université de Nice Sophia Antipolis, UMR 1301, 400 route des Chappes, F-06903 Sophia Antipolis, France, 4Lab. Nematologia/ICAM, Dept. Biologia, Universidade de Évora, 7002-554 Évora, Portugal, 5Department of Plant Systems Biology, VIB, Ghent, Belgium.

Plant-parasitic nematodes of the genera *Meloidogyne* are capable to induce giant cells that undergo repeated mitosis without cytokinesis possibly alternated with endoreduplication cycles. Promoter activity and mRNA localization of key cell cycle genes like *CDKA;1*, *CDKB1;1*, *CYCB1;1*, and *CYCA2;1* showed early induction of these genes in both nematode feeding site (NFS). In addition, disturbance in NFS development and nematode maturation were observed during treatment of infected roots with cell cycle inhibitors. DNA synthesis experiments demonstrated that both feeding sites undergo extra endocycles possibly justifying the large nuclei present in NFC. How precisely nematodes manipulate the cell cycle in their favor remains to be understood. A systematic comparison of the temporal and spatial expression pattern of different classes of core cell cycle genes between uninfected roots and nematode infected *Arabidopsis thaliana* plants resulted in the identification of a collection of genes possibly implicated in NFC development. Among them, one member of the so-called interactors of cyclin-dependent kinase/Kip-related proteins (ICK/KRP), negative regulators of the cell cycle, showed to be upregulated during NFS development. Recent work has shown that KRP2 might regulate mitosis-to-endocycle transition during Arabidopsis leaf development and is highly expressed in endoreduplicating cells as potentially occurring in

nematode feeding cells KRPs. To address the direct relevance of these cell cycle inhibitors genes for NFS ontogeny, mutant lines over- expressing and knocking-down are being used to determine how NFS development is affected, and which family members are potential involved in the NFS formation. Furthermore, *in vivo* subcellular localization of these cell cycle proteins in NFS has been followed to understand the dynamics of these proteins during giant cell development. Based on our preliminary results, some of these cell cycles inhibitors genes are promising candidates involved in NFS development.

ROOT-KNOT NEMATODE FEEDING SITE DEVELOPMENT IS IMPAIRED BY CYCLIN-DEPENDENT KINASE INHIBITORS. **Vieira, Paulo[1,2,3,4], G. Engler[1,2,3], M. Mota[4], P. Abad[1,2,3], L. Veylder[5], and J. Almeida-Engler[1,2,3].** [1]INRA, UMR, 1301, 400 route des Chappes, F-06903 Sophia Antipolis, France, [2]CNRS, UMR 6243, 400 route des Chappes, F-06903 Sophia Antipolis, France, [3]Université de Nice Sophia Antipolis, UMR 1301, 400 route des Chappes, F-06903 Sophia Antipolis, France, [4]Lab. Nematologia/ICAM, Dept. Biologia, Universidade de Évora, 7002-554 Évora, Portugal, [5]Department of Plant Systems Biology, VIB, Ghent, Belgium.

Plant-parasitic nematodes of the genera *Meloidogyne* trigger the formation of giant cells that undergo recurring acytokinetic mitosis and endocycles. Expression analyses of key cell cycle genes showed their early induction in the nematode feeding site (NFS). Additionally, disturbance in NFS development and juvenile maturation were observed during treatment of infected roots with cell cycle inhibitors. Intense DNA synthesis and enlarged nuclei demonstrated that giant cells undergo additional endocycles. How precisely nematodes manipulate the cell cycle in their favour remains to be understood. A systematic comparison of the temporal and spatial expression pattern of core cell cycle genes between uninfected roots and in galls of *Arabidopsis thaliana* resulted in the identification of a collection of genes up- or downregulated in NFC. Among them, negative regulators are candidates to control the cell cycle in NFC. Previous work has shown that KRP2, a member of the cyclin-dependent kinase/kip-related proteins (ICK/KRP), regulate mitosis-to-endocycle transition in plant cells, and is expressed in endoreduplicating cells. The KRP2 gene showed to be expressed during gall development. Therefore to study the relevance of the KRP cell cycle inhibitor genes (7 in *Arabidopis*) for NFS ontogeny, mutant lines over-expressing and knocked-out are being tested to determine their effect on feeding site development. *In vivo* subcellular localization studies have been carried out to better understand the dynamics of these proteins during giant cell development. Based on these data, three KRP genes are perceived to control giant cell size and consequently nematode reproduction.

PLANT GROWTH AND HEALTH PROMOTION FOLLOWING ENHANCEMENT OF TOMATO WITH MUTUALITISTIC FUNGAL ENDOPHYTES WHEN CHALLENGED BY THE ROOT-KNOT NEMATODE *MELOIDOGYNE INCOGNITA*. **Vu[1], Tam T. and R. A. Sikora[2].** [1]Institute of Ecology and Biological Resources. Vietnam Academy of Science and Technology. 18 Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam (VAST) and [2]Soil-Ecosystem Phytopathology and Nematology. INRES - Department of Phytomedicine, University of Bonn. Nussallee 9, 53115 Bonn, Germany.

Fifteen non-pathogenic endophytic *Fusarium oxysporum* strains were isolated from the endorhiza of surface sterilize tomato roots from different areas of northern Vietnam. The strains were tested for tomato growth promotion in the greenhouse. Six weeks after the seedlings were inoculation with the endophytes, fresh shoot and root weight increased significantly 18-32 percent in plants treated with six of the strains. In additional tests, *M. incognita* root galling and number of egg masses were reduced 34-55% six weeks after nematode inoculation on plants treated with eight of the *F. oxysporum* isolates. Five of the endophytes simultaneously demonstrated significant plant growth and health promoting effects in tomato. These endophytes when used to treat seedling prior to transplanting into the field could prove to be an additional tool in the integrated management of root-knot nematode *M. incognita* in tomatoes.

POST-PLANT NEMATICIDES FOR THE CONTROL OF *PRATYLENCHUS PENETRANS* IN RED RASPBERRY. **Walters[1], Thomas W., J.N. Pinkerton[1*], and I.A. Zasada[2].** [1]Washington State University Northwest Research and Education Center, Mount Vernon, WA 98273 and [2]USDA-ARS Horticultural Crops Research Laboratory, 3420 NW Orchard Ave., Corvallis, OR 97330.

Red raspberries (*Rubus ideaus*) are a major crop in the Pacific Northwest of the US, with this region producing over 90% of the nation's processed raspberries. *Pratylenchus penetrans* is commonly found in raspberry plantings and has been shown to reduce raspberry vigor and yield. Currently, there are no effective post-plant nematicides labeled for use in raspberries to control this plant-parasitic nematode. To identify a post-plant nematicide to control *P. penetrans* in raspberry, several commercially-available nematicides were tested in greenhouse and field trials. Promax and NemaQ were tested in both experimental venues, while Movento and Cordon were tested in the field trial only. Nemacur was included in the field trial as the industry standard which is no longer registered for

use on raspberry, and Vydate which is labeled on raspberry in Canada, but not in the US was included in the greenhouse trials. In all trials a nontreated control was included; in the greenhouse trials a noninoculated, nontreated control was also included. All treatments were replicated 6 to 8 times and appropriately randomized and blocked. In the greenhouse trials, one-month-old raspberries 'Meeker' were inoculated with 3,000 *P. penetrans*. One month later nematicides were applied as soil drenches, and NemaQ and Promax were applied four times as three-week intervals. Plants were destructively sampled after three months. Only Vydate reduced the number of *P. penetrans*/50 g soil and /g root compared to the nontreated control. None of the nematicides were phytotoxic with shoot and root weights similar to the noninoculated, nontreated control. The field trial was conducted in an established raspberry 'Meeker' planting with initial average fall population densities of 940 *P. penetrans*/g root. Nemacur was applied in the fall and all other nematicides were applied in the spring. Cordon was applied once as a drip, Monvento was applied to the foliage, and Promax and NemaQ were applied directly to soil four times at two-week-intervals followed by 10 mm of water. *Pratylenchus penentrans* population densities were evaluated during harvest (July) and after harvest (October), and fruit was harvested three times during the season to assess yield. On both sampling dates, none of the nematicides reduced *P. penetrans* populations in soil or roots compared to the nontreated control. There was no effect of nematicides on raspberry yield. At the rates and timings used here, none of the tested nematicides (except for Vydate) reduced *P. penetrans* population densities or enhanced raspberry growth or productivity.

DEVELOPMENT OF A MOLECULAR METHOD FOR NEMATODE COMMUNITY ANALYSIS IN HAWAII. **Wang*, I-Chin, K.-H. Wang and B.S. Sipes.** Department of Plant Environmental Protection Sciences, University of Hawaii at Manoa, Honolulu, Hawaii, 96822, USA.

Soil sustains a variety of biota while maintaining and enhancing the quality of life, water and air. Plant and animal productivity can be greater in healthy soil ecosystems compared to unhealthy soils. In order to ensure sustainability in agricultural soil ecosystems, assessment of soil health is required. Nematodes are good indicators of the structure (*c-p* value) and function (different feeding groups) of the soil ecosystem, and provide a reference for soil health conditions. However, performing nematode community analysis via microscopic analysis is laborious and technically challenging. Our objective is to develop a molecular tool that can potentially replace conventional visual nematode community analysis. We approach the nematode community analysis utilizing qPCR with Taqman probe. Known quantities of selected representative nematode families within different nematode functional guilds were subjected to qPCR and nematode community indices calculated. qPCR primers have been designed to the conserved 18S rDNA region. Primer specificity was determined computationally with the Genbank database and with a PCR assay. Genbank database alignment has shown specificity of each primer set within and between nematode guilds. The PCR assay revealed specificity for the bacterivore, fungivore, omnivore and predator primer sets. Overall, these qPCR primer sets are qualified for use in a qPCR assay of soil health which is fast and accurate.

ALLELOPATHIC EFFECTS OF *CROTALARIA* SPP. AGAINST MELOIDOGYNE INCOGNITA AS AFFECTED BY CROP AGE, PLANT PART, BIOMASS, HEAT, AND SPECIES. **Wang, Koon-Hui[1], and Zasada, I.A.[2].** [1]Department of Plant and Environmental Protection Sciences, University of Hawaii at Manoa, 3050 Maile Way, Honolulu, HI 96822; USDA-ARS, 3420 NW Orchard Avenue, Corvallis, OR 97330.

Although sunn hemp, *Crotalaria juncea* (Cj), is known to suppress *Meloidogyne* spp. when grown as a cover crop and soil incorporated, variable levels of suppression do occur in the field. Based on 10 field trials in Hawaii, biomass of Cj varied from 0.73 to 6.67 Mg/ha of dry Cj residues over approximately a 2-mon growing period. This variation could be seasonal or due to soil pH. To begin to understand the variability in *Meloidogyne* spp. suppression by Cj, controlled laboratory experiments were conducted to understand the influence of crop age, plant part, and concentration of Cj on *M. incognita* suppression. Additional objectives were to examine if 1) integration of Cj and solarization (Sol) could increase nematode suppressive effect of Cj, and 2) different species of *Crotalaria* [*C. juncea, C. spectabilis* (Cs), and *C. retusa* (Cr)] might be more toxic to *M. incognita*. Tissues of Cj were collected at 1, 2, 3, and 4 mon after planting and partitioned into stem, leaves, flowers, and roots or kept as whole. Each testing unit was prepared as 0.1, 0.5, 1.0, and 2.5% w/v leachates in water. Juveniles (J2) of *M. incognita* were introduced into designated leachates for 48 hours and then transferred to water for another 24 hours; suppression of J2 was determined at both times. Eggs were exposed in designated leachates for one week and percentage hatch determined. Based on factorial analysis of variance, Cj suppressed *M. incognita* J2 more efficiently than egg hatch. One- and 3-mon old tissues were more suppressive to J2 and egg hatch than 2- and 4-mon old tissues. Although leaf tissue was most suppressive to J2 when directly exposed to the leachate, flower or whole plant tissues were more suppressive to J2 than leaf even after transferring to water. Leaf tissue was least suppressive part to egg hatch. Significant interaction among Cj age, part, and leachate concentration occurred ($P < 0.01$). Most Cj tissues

inactivated 100% J2 after 48-hr incubation at 2.5% concentration, but all ages of leaf tissue and 4-mon old whole plant tissue inactivated 100% J2 at ≥ 1% concentration, whereas 3-mon old flower tissue killed 100% J2 at ≥ 0.5%. In field trials, integrating Cj+Sol did not increase nematode suppressive effects on plant-parasitic nematodes as compared to Cj alone when the solarization was conducted during summer. Among the three species examined, Cj was most suppressive to *M. incognita* J2 and egg hatch, followed by Cs and Cr ($P < 0.05$). However, an interaction among species, plant parts, and concentrations was observed ($P < 0.01$). Allelopathic effects of Cj, Cs, and Cr were nematostatic rather than nematicidal, except for 1- and 3-mon Cj flower, 1-mon Cj leaf, and 4-mon Cs roots.

INVESTIGATIONS OF LEGUME RESISTANCE REACTIONS TO HETERODERA GLYCINES IN ONTARIO FIELDS. **Welacky, Tom.** Greenhouse and Processing Crops Research Centre, Agriculture & Agri-Food Canada, 2585 County Road 20, Harrow, ON. NOR 1GO, Canada.

A preliminary investigation was conducted on Heterodera glycines root reproduction in a field situation on soybean and edible beans in Ontario, Canada. Screening of legumes for reproduction of nematodes is generally carried out under controlled conditions in pots with arranged levels of soybean cyst nematode (SCN) numbers, water temperature and soil type in a greenhouse environment. Field testing is an uncommon method for screening due to the variable biotic and weather factors of the field environment influencing SCN reactions that may result in false or inconsistent results. It was decided to test the possibility of screening varieties and HG Types in monitored regional fields that had uniform SCN populations and soil types over two, 4 year periods. Testing was carried out on well drained loamy sand plots (65-70% sand and less than 10% clay). In addition to root counts, legumes were sampled for resident SCN cyst and egg populations with a probe on each side of the row at planting and at harvest. At 35-40 days after planting, roots were extracted with spade and or a tractor mounted tree nursery digger to a depth of 30-35 cm. Plants were removed in groups with as much undisturbed soil as possible and placed in 4 litre pails lined with plastic bags. Pails were transported to the extraction lab and soaked in water for 1-2 hours. Plants were carefully separated and individual roots were rinsed and then power washed over a set of nested sieves. Cysts and eggs were collected and counted. Field testing of varieties indicated a there was a significant correlation between root weights and number of cysts per root four out of five years and for HG Type testing two out of five years. Single year comparisons of greenhouse to field screening indicated a significant correlation for the number of cysts on the roots in the field to the number of cysts per root tested in the greenhouse and two field locations. The analysis and correlation of results from a multiple number of factors across time periods as well as a preliminary comparison to greenhouse screening methods will be presented.

EFFECT OF ENVIRONMENTAL PARAMETERS ON EFFICACY OF ALDICARB AND COTTON SEED NEMATICIDE TREATMENTS. **Wheeler, Terry A. and B.G. Mullinix, Jr.** Texas AgriLife Research, 1102 E. FM 1294, Lubbock, TX 79403.

Nematicide trials were conducted in 2009 and 2010 at three irrigation rates, where irrigation rate (base (B), B-33%, B+33%) was the main plot and nematicide treatments were the subplots. Nematicide treatment (aldicarb or nematicide seed treatments) yielded significantly higher than the nontreated check with the B irrigation rate, however there were no treatment effects on yield at the B-33% and B+33% irrigation rates. A combination of 20 tests conducted over 7 years at the same root-knot nematode (*Meloidogyne incogntia*) infested field was examined for impact of environmental variables and nematicide treatments on root galling around 35 days after planting and yield. Root galling was significantly lower when aldicarb was applied than for the nontreated check or when nematicide seed treatments (abamectin or thiodicarb) were applied. When total water applied in May and average high temperature in May were used as covariates, then root galling was highest under a low cumulative water in May and when average high temperatures were low. As cumulative water in May increased, then a greater reduction in root galling was seen for both the nematicide seed treatments and aldicarb relative to the nontreated or insecticide treated checks. Yield was higher for plots treated with aldicarb compared to the nontreated check when cumulative water in August and July and heat units in July (all interacting with treatment) were included in the model. Nematicide treated plots yielded the highest when August was very wet and cumulative water through July was low. Aldicarb treated plots yielded much higher than seed treatment nematicides in this scenario. Seed treatment nematicides were as effective as aldicarb only under a narrow range of environmental conditions.

HOST RESISTANCE AS A NEMATODE MANAGEMENT TOOL. **Williamson, Valerie M. and V.P. Thomas.** Dept. of Nematology, University of California, Davis 95616.

When available, host resistance is a desirable means to control pathogen damage in crop plants. Nematode resistance has been identified in many crop species or has been introgressed from wild species. In some cases the responsible genes have been genetically mapped, and, in a few cases, they have been cloned. The molecularly characterized nematode resistance proteins belong to a large group of pathogen resistance proteins that act as

surveillance molecules, recognizing the presence of specific pathogens and triggering host defense. Management of resistance by such genes can be complex due to the selection of variant pathogens that circumvent recognition. The *Mi-1* gene of tomato, which confers effective field resistance to three important species of root-knot nematode (RKN), *Meloidogyne incognita*, *M. javanica* and *M. arenaria,* is one of best-studied examples of host resistance to nematodes. This gene is widely deployed in processing tomato varieties in California and is generally effective at controlling RKN. However, in recent years galling has been noted on tomato with *Mi-1* in several locations throughout the state. Greenhouse assays of samples collected from diverse locations in California confirmed that the galling was due to *Mi-1*-virulent RKN. Molecular characterization of the virulent nematodes identified most as *M. incognita* or *M. javanica* although one isolate may be a species not previously characterized in California. Attempts to identify molecular markers that differentiate nematode isolates that are virulent from those that are avirulent on *Mi-1*-tomato have not been successful. Currently *Mi-1* is the only source of nematode resistance in cultivated tomato. The broad host range of RKN has made it difficult to identify appropriate non-host rotation crops for managing resistance-breaking nematodes. Resistant varieties of susceptible crops are a potential option for rotation. We have evaluated potential rotation crops using our collection of resistance-breaking nematodes. Wheat is a common rotation crop used in California, but has been reported to be susceptible to RKN. Surprisingly, we found that some wheat varieties are highly resistant to all RKN isolates tested due to an alien introgression that carries resistance to stem rust. Roots of resistant wheat varieties are attractive to RKN and nematodes are able to enter, but there is little reproduction. Such wheat varieties may be a useful rotation to control resistance–breaking nematodes. Of the other hosts tested, the bell pepper variety Carolina Wonder and NemX cotton showed reduced reproduction of *Mi*-virulent nematodes, but none were as consistent at lowering nematode numbers as the resistant varieties of wheat. One variety of lettuce showed resistance to some, but not all, *Mi*-virulent nematode strains.

OBSERVATIONS ON A ROOT-KNOT NEMATODE IN COACHELLA VALLEY TURF. **Witte[1], Hannes, A. Ploeg[1], S. Subbotin[2], I. DeLey[1], J. Smith Becker[1], and J.O. Becker[1].** [1]Department of Nematology, University of California, Riverside, CA 92521, [2]Plant Pest Diagnostics Branch, California Department of Food and Agriculture, Sacramento, CA 95832.

In September 2009, a private golf club near Palm Desert alerted Nematologists from the University of California, Riverside to severe signs of decline in their bentgrass (*Agrostis palustris* cv Penn A-4) greens. None of the Bermuda grass fairways and roughs (*Cynodon dactylon* cv Tiff) or landscape areas appeared to have similar problems. Spurge (*Euphorbia* spp.) and purslane (*Portulaca* spp.) as well as Bermuda grass from the fairways were found invading the declining bentgrass greens. Soil and root samples obtained from affected areas contained several hundred root-knot nematodes per 100 $cm^3$ soil. Root-knot nematodes were also present in the fairways but not in landscape plantings. Bentgrass roots showed characteristic galls with light to dark brown discoloration indicating advanced stages of root senescence that are typically caused by secondary infection with fungi and bacteria. A population raised from a single egg mass was used for further laboratory and greenhouse studies. The nematode completed its life cycle in excised corn root culture. Preliminary host range tests revealed an exclusive preference for monocotyledons, in particular Poaceae. Based on temperature bath studies, base temperature and heat sum were estimated at 8.4°C and 500 degree-days, respectively. At an optimum temperature of 29°C the life-cycle of the nematode was completed in approximately 24 days. Greenhouse studies with increasing population densities of the root-knot nematode did not result in significant growth reductions in 'Penn A-4' bentgrass despite causing abundant root galling. Temperature stress and/or interactions with fungal pathogens may have led to the observed decline on the golf course green. Morphological and molecular identification attempts have been so far inconclusive although mitochondrial DNA phylogenetic analysis suggested a close relationship to *M. graminis*.

IDENTIFICATION OF FIVE FUNGAL SPECIES ISOLATED FROM NON-VIABLE EGGS OF A SINGLE *GLOBODERA PALLIDA* CYST BASED ON A POLYPHASIC APPROACH. **Worapong[1], Jeerapun, T. Johnson[2], X. Gao[1], J. Kuhl[1], C. Bates[3], J. B. Johnson[1] and R. S. Zemetra[1].** [1]Department of Plant, Soil and Entomological Sciences, University of Idaho, Moscow, Idaho 83844-2339, [2]Department of Molecular Biology and Biochemistry, Life Science 142, P.O. Box 443052, Moscow, ID 83844-3052, and [3]Department of Plant Pathology, Washington State University, PO Box 646430, Pullman, WA 99164-6430.

*G. pallida* cysts have been known to persist in soil for long periods of time, and have been found to have high levels of resistance to chemical treatments. Biological control agents, an environmentally friendly approach to non-target organisms, are a potentially valuable management tool because they last throughout the season once established in a nematode population. To determine if fungal biocontrol agents were present in the *G. pallida* population in southern Idaho, potential egg pathogenic fungi of *G. pallida* were isolated from non-viable eggs of individual cysts collected from infested fields. Interestingly, a single cyst that contained only non-viable eggs based

on Meldola's blue staining provided five different fungal colonies on potato dextrose agar. These five fungi were isolated into pure cultures using a hyphal tip technique for species identification based on a polyphasic approach. Based on morphological characteristics and ITS sequences, the fungal species were identified as *Fusarium tricinctum* strain ADE1, *F. oxysporum* strain ADE2, *F. solani* strain ADE3, *Paecilomyces lilacinus* strain ADE4 and *Plectosphaerella cucumerina* strain ADE5. Included in this work, the ITS sequences of the fungal isolates were analyzed and collected for a molecular database that will be developed for identification and monitoring of the selected biological control agents of *G. pallida* in greenhouse and field trials. Phytopathogenicity tests of the isolated fungi showed that they were not pathogenic to Désirée and Russet Burbank potato cultivars after two months of inoculation under greenhouse conditions. These results imply that the above isolates may have potential for use as biocontrol agents of *G. pallida*. Further research of antagonistic tests will be conducted to study the effect of these fungi on viability and hatching of *G. pallida* both *in vitro* and in plant experiments in the greenhouse.

A NEWLY DESCRIBED *DIPLOLAIMELLA* SPECIES WITH RED PIGMENTED OCELLI HAS POTENTIAL FOR USE IN PESTICIDE DETECTION. **Wu, Hsiu-Chen[1], Y.C. Chen[1*], P. Chen[1] and T. T. Tsay[1].** [1]Dept of Plant Pathology, National Chung-Hsing University, 250 Kuo-Kuang Rd. Taichung 402, Taiwan.

A population of fresh water and free-living nematode was isolated from Yunchiada Bridge in Beigang River Basin in Yun-Lin County, Taiwan. Based on the morphological data of our study, this nematode was classified as *Diplolaimella* species. The stoma was divided into 2 chambers with no denticles inside. Ocelli consisted of sensory body and red pigment cup were located dorso-laterally on epidermis cell. Amphids were circular. Pharynx was cylindrical and the diameter enlarged gradually in the posterior region. Male had distinct spicules and gubernaculums and its tail tapered gradually to the round spinneret. No reported *Diplolaimella* species had de Man's morphometrical measurements similar to the one found in this study. Sixteen pesticides including insecticides, herbicides and fungicides were used to test the coloration of the pigment cup on this *Diplolaimella* species. The red pigment cup lost its color when treated with 100 ppm of Ethoprop, Butachlor, Parquat, Pendimenthain, Glyphosate isopropylamine or Chlorothalonil. The red color of the pigment cup in *Diplolaimella* species did not change when treated with pH values ranging from 4 to 10, or doubly charged heavy metals including An, Mg, Hg, Cu, Mn and Cd. Several pesticides such as Carbofuran and Oxamyl caused 100% mortality of *Diplolaimella* species but did not affect the coloration of the red pigment cup, indicating that the lost of red pigment is not correlated with the nematode mortality. The mechanism causing the lost of the red pigment is under investigation.

BELOW GROUND NATURAL BIOCONTROL SERVICES IN A POST-INDUSTRIAL URBAN ECOSYSTEM. **Yadav, Priyanka, K. Duckworth, and P. S. Grewal.** Center for Urban Environment and Economic Development, Department of Entomology, The Ohio State University, OARDC, 1680 Madison Avenue, Wooster, OH 44691, USA.

Urban agriculture offers a framework for local self-reliance in disadvantaged neighborhoods, particularly in post-industrial cities, by providing food security, employment opportunities, and other community benefits. However, urban agriculture relies on the supporting and regulating services of the soil food web which may be affected by anthropogenic activities in urban ecosystems. In this context, the extent of natural pest control services provided by the soil foodweb in urban ecosystems is an unexplored frontier. We quantified the below ground natural biocontrol services in urban gardens and vacant lots in two post-industrial cities, Akron and Cleveland (Ohio, USA), over a two-year period using an in-situ insect baiting technique. Natural below ground biocontrol activity in vacant lots and urban gardens varied between 51% and 98% with major contributions by ants, microbial pathogens, and entomopathogenic nematodes. Ants showed significantly higher ($p < 0.0001$) biocontrol activity in vacant lots (60% ± 33.4%) than in urban gardens (33.3% ± 22.2%) whereas microbial pathogens exhibited significantly higher ($p < 0.0001$) activity in urban gardens (27.8% ± 15%) than vacant lots (8.3% ± 16.7%). Newly established garden sites exhibited slightly but not significantly lower biological control activity (67% ± 15%) than the older urban gardens (78% ± 18%). The high inherent biological control activity observed in this study indicates the resilience of the soil food web in urban ecosystems, which can serve as a foundation for designing ecological landscaping practices to enhance urban environment and boost local self-reliance in food.

DEVELOPING A SPECIES-SPECIFIC PCR FOR DETECTING *HETERODERA AVENAE* IN PACIFIC NORTHWEST SOILS. **Yan, Guiping[1], R.W. Smiley[1], and A. Skantar[2].** [1]Oregon State University, Columbia Basin Agricultural Research Center, Pendleton, OR 97801; [2]USDA-Agricultural Research Service, Nematology Laboratory, Beltsville, MD 20705.

The cereal cyst nematode *Heterodera avenae* is an important sedentary plant-parasitic nematode that restricts production of cereal crops worldwide. In the USA, this nematode is known to occur in at least seven western states

including three Pacific Northwest (PNW) states; Oregon, Washington, and Idaho. High populations of *H. avenae* reduced yield of intolerant wheat cultivars as much as 50 percent. It was estimated that this nematode reduces the profit from wheat production by at least $3.4 million annually in the PNW. Accurate detection and identification of *H. avenae* in infested fields are critical for recommending and implementing effective management practices. However, based on morphological features it is difficult and time-consuming to distinguish this nematode from other closely related *Heterodera* spp., particularly *H. filipjevi*, another economically important cereal cyst nematode that is known to be present in Oregon wheat fields. A species-specific PCR assay was developed to facilitate detection and identification of this nematode. Species-specific primers were designed from the internal transcribed spacer (ITS) region of *Heterodera* rDNA gene sequences. The primers were tested with DNA of nine *Heterodera* spp., four *Globodera* spp., five *Meloidogyne* spp., four *Pratylenchu*s spp., and three other plant-parasitic and non-parasitic nematodes and six fungal pathogens typically present in the PNW wheat fields. A primer set produced a specific amplicon (242 bp) from target populations of *H. avenae* collected from Oregon, Washington and Idaho, and did not produce any specific amplification from the above non-target control species. The primer pair was mixed with *H. filipjevi*-specific PCR primers developed in our laboratory and both species were identified with different band profiles in the same PCR reaction. Optimum PCR reaction and amplification conditions were established. Robust PCR amplification was achieved with DNA extracted from a single egg or second-stage juvenile using typical proteinase K buffer. Different numbers of eggs or juveniles were added separately to non-infested, sterilized soil and DNA was extracted directly from soil using a commercial kit (PowerSoil DNA Isolation Kit). This PCR assay was capable of detecting a single egg or second-stage juvenile in 1 g of sterilized soil. The new PCR method is being tested for application in a range of field soils naturally infested with *H. avenae* in the three PNW states. The species-specific primers are also currently being examined for use in a real-time PCR system for quantification of *H. avenae* from infested field soils.

DEVELOPMENT OF PRIMETIME-REAL-TIME PCR FOR SPECIES IDENTIFICATION OF SOYBEAN CYST NEMATODE (*HETERODERA GLYCINES* ICHINOHE, 1952) IN NORTH CAROLINA. **Ye[1], Weimin.** [1]Nematologist, Section Chief, Nematode Assay Section, Agronomic Division, North Carolina Department of Agriculture & Consumer Services, Raleigh, NC 27607.

Soybean cyst nematode (SCN) is an obligate, sedentary parasite that is a major pathogen of soybean and accounts for an estimated 1 billion dollars in production losses annually in the United States of America. This paper describes the development of a real-time PCR method for rapid, sensitive, species-specific and accurate identification of SCN alone or on mixed populations with other nematodes in North Carolina. The 83-bp DNA fragment of PrimeTime-real-time PCR was designed based on a 477-bp-SCN-SCAR marker previously proved to be SCN-specific. A total of 44 populations including cyst forming nematodes (*Heterodera glycines*, *H. fici*, *H. schachtii*, *H. trifolii*, *Cactodera weissi*, *Globodera tabacum*, *Meloidodera floridensis* and other unidentified cyst nematodes) and non-cyst forming nematodes (*Ditylenchus dipsaci*, *Meloidogyne incognita* and *Xiphinema chambersi*) were tested in this study, all SCN populations are tested positive and non-SCN populations negative. This assay for the detection and identification has been successfully applied for testing a single SCN cyst, a 2$^{nd}$-stage-SCN juvenile, a single SCN egg, up to ten SCN cysts, a 10-fold dilution of a single 2$^{nd}$-stage-SCN juvenile and 20-fold dilution of one SCN cyst. The assay is not SCN-race specific. It gave an accurate positive result when SCN is mixed with other cyst species. Besides, nematode universal primers/probes for real-time PCR amplification as a nematode endogenous control to detect the presence of 18S ribosomal RNA (rRNA) gene were employed in this assay, so that a SCN-negative sample can be tested to exclude false negative. This method will be very useful for a broad range of research programs as well as the regulatory response and management of SCN in North Carolina and other region of the southeastern U.S.A.

EFFECTS SOIL FUMIGANTS ON *MELOIDOGYNE CHITWOODI* AND FREE-LIVING NEMATODES IN PACIFIC NORTHWEST POTATOES. **Yoshida, Harvey[1], J. Wilson[3], J. Busacca[4].** [1]Dow AgroSciences, Richland, WA, [2]Washington State University, Prosser, WA, and [3]Dow AgroSciences, Indianapolis, IN.

Soil fumigants are a key component for the management of plant parasitic nematodes in potatoes within the Columbia Basin of Oregon and Washington. Infection by the Columbia rootknot nematode, *Meloidogyne chitwoodi*, can cause direct damage to potato tubers resulting in significant reductions in crop quality and yield. Multi-year research trials have demonstrated the effectiveness of 1,3-dichloropropene (Telone® II at 20 gallons/acre) and the combination with metam sodium (Telone® II at 15 gallons/acre + metam sodium at 30 gallons/acre) against *M. chitwoodi*. Results also show that while there is a reduction in free-living nematode densities immediately following fumigation, populations at harvest are equal to or higher than pre-fumigation levels. These results suggest that 1,3-dichloropropene and metam sodium remain effective tools for the management of *M. chitwoodi* and their impact on free-living nematode species are only transient in nature.

IDENTIFICATION OF COMMONLY ENCOUNTERED *PRATYLENCHUS* IN OREGON. **Zasada, Inga A.[1], A. Peetz[1*], N. Wade[2], and R.E. Ingham[2].** [1]USDA-ARS Horticultural Crops Research Laboratory, 3420 NW Orchard Ave., Corvallis, OR 97330 and [2]Department of Botany and Plant Pathology, Cordley 2082, Oregon State University, Corvallis, OR 97330.

*Pratylenchus* species are commonly encountered in soil samples collected from a diversity of economically important crops in Oregon including potato and small fruits. Proper identification is critical to the selection of an appropriate management strategy since, in many cases, populations are an assemblage of more than one *Pratylenchus* spp. that vary in pathogenicity. Identification of *Pratylenchus* species using differences in morphological characteristics can be time consuming because differences between species can be quite subtle. Furthermore, identification usually requires adult specimens, but often the populations recovered from soil are predominately juveniles. Therefore, the development of a reliable molecular technique to differentiate between the species commonly encountered in Oregon, *P. penetrans*, *P. neglectus*, *P. thornei*, and *P. crenatus*, would be highly desirable. To this end, a novel multiplex PCR method for the detection of these commonly encountered *Pratylenchus* spp., as well as *P. vulnus* which is less common, was developed. Previously published and newly developed species-specific primers were tested for functionality and specificity in single reactions prior to combining them in a multiplex PCR. A multiplex PCR assay was designed and optimized to differentiate between *P. penetrans*, *P. neglectus*, *P. thornei*, *P. crenatus*, and *P. vulnus* with PCR products of 65, 144, 288, 371, and 186 bp, respectively. The utility of this multiplex PCR method will be tested by assessing the diversity of *Pratylenchus* spp. that occur in Oregon blueberry fields and for the detection of *P. penetrans* which rarely occurs in Oregon potatoes, but can be highly pathogenic. Ultimately, this diagnostic multiplex PCR assay could be used as an efficient tool for rapid analysis of soil samples in any laboratory equipped for PCR.

DURABILITY OF NEMATODE RESISTANCE IN GRAPE ROOTSTOCKS. **Zheng[1], Liang, H. Ferris[1], and M.A. Walker[2].** [1]Department of Nematology, University of California, Davis, CA 95616, USA and [2]Department of Viticulture and Enology, University of California, Davis, CA 95616, USA.

We recently released five grape rootstocks, UCD-GRN1 with *Vitis rupestris* and *Muscadinia rotundifolia* parentage, UCD-GRN2 with *V. riparia, V. rufotomentosa* and *V. champinii* Dog Ridge parentage, UCD-GRN3 and UCD-GRN4 with *V. riparia*, *V. rufotomentosa*, *V. champinii* Dog Ridge, and *V. champinii* c9038 parentage, and UCD-GRN5 with *V. riparia, V. champinii* Ramsey, and *V. champinii* c9021 parentage. Between 2003 and the present, we have tested the durability of resistance in these and other rootstocks, and their parents, to root-knot nematodes when challenged by higher nematode populations, combinations of nematode species, and at a range of soil temperatures. At 25°C soil temperature, resistance to several populations of *Meloidogyne incognita* and *M. arenaria* was not affected by increase in nematode density from 1,500 to 10,000 per pot. The resistance to root-knot nematodes was not compromised when they were in combination with *Xiphinema index*, *Criconemoides xenoplax* or *Tylenchulus semipenetrans*. In several series of experiments in soil temperature tanks, strains of *M. incognita* and *M. arenaria* that are virulent on the resistant Harmony rootstock, as well as *M. incognita* Race 3 which is avirulent on Harmony, failed to produce egg masses on the UCD-GRN series rootstocks and other resistant selections at temperatures below 25°C. At 27°C and above we observed increases in prevalence and abundance of nematode galling and egg mass production with temperature. At 30°C, egg mass production on the UCD-GRN series was 5%, and on the widely-used Harmony rootstock was 12%, of that on the susceptible control. At 32°C, nematode reproduction was apparently suppressed by temperature because, in many cases, it was less than at 30°C on both susceptible and resistant rootstocks. The susceptibility of their parents responded in a similar manner to temperature as the resistant rootstock selections. We determined the effect of soil temperature on the reproduction and survival of the ring nematode, *C. xenoplax*, on rootstock selections with and without *M. rotundifolia*, our source of resistance for ring nematode, in their parentage. Although ring nematode reproduction was suppressed by 50% at 30°C on susceptible cultivars, pure *M. rotundifolia* and the resistant selections (UCD-GRN1 and O39-16) maintained their resistance and there was almost no survival or reproduction of the nematode after 3 months of exposure at soil temperatures between 24 and 33°C. We conclude that the newly-release rootstocks will perform well in coastal and northern areas of California but that there is a possibility of some root-knot nematode reproduction in inland regions with seasonally higher soil temperatures.