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ABSTRACTS

PCR-DGGE AS A TOOL FOR IDENTIFICATION OF *MSP1* GENE VARIANTS IN POPULATIONS OF *MELOIDOGYNE INCOGNITA*. **Adam, M.[1,2], J. Hallmann[1], and H. Heuer[1].** [1]Julius Kühn-Institut, Federal Research Centre for Cultivated Plants (JKI), Institute for Epidemiology and Pathogen Diagnostics, Messeweg 11/12, 38104 Braunschweig, Germany; [2]Department of Zoology and Nematology, Cairo University, Giza, Egypt.

The Root-knot nematode *Meloidogyne incognita* is one of the most economically important pests causing severe damages and losses in a wide variety of crops. Its management using resistant plant cultivars or appropriate crop rotations is an effective and environmentally friendly method. However, this strategy requires an accurate and rapid discrimination of the populations regarding virulence on resistant cultivars or crop-/cultivar dependent aggressiveness. Here we report development of a polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) as a molecular tool to detect differences in DNA sequences or mutations of various genes to compare the distribution of variants of the effector gene *msp1* between seven populations/races originated from different countries. Sequencing of cloned PCR products revealed five *msp1* variants from these populations which were distinguishable in their reproduction on five host plants. PCR-DGGE facilitated the discrimination among these populations and/or races based on these msp1 variants that were detected by five clear bands at different positions. DGGE for replicated pools of juveniles from the seven populations revealed ten variants of *msp1*. A correlation between the presence of a particular gene variant and the reproductive potential on particular hosts was not evident. Especially race 3 showed substantial variation within the population. DGGE fingerprints of *msp1* tended to cluster the populations according to their reproduction rate on pepper. The developed method could be useful for analyzing population heterogeneity and epidemiology of *M. incognita*.

THE IDENTITY AND REPRODUCTION POTENTIAL OF SOUTH AFRICAN *MELOIDOGYNE* SPECIES. **Agenbag, M.[1], H. Fourie[1], C.M. Mienie[1], M. Marais[2], M. Daneel[3], and G. Karssen[4].** [1]North-West University, School of Environmental Sciences and Development, Private Bag X6001, Potchefstroom 2520, South Africa, Agricultural Research Council-Plant Protection Research Institute; [2]Nematology Unit, Biosystematics Division, Private Bag X134, Queenswood 0121, South Africa; [3]Agricultural Research Council – Institute for Tropical and Subtropical Crops, Private Bag X11208, Nelspruit, 1200, South Africa; [4]Wageningen UR Plant Sciences, Laboratory of Nematology, PO Box 8123, 6708PB, Wageningen, The Netherlands.

Root-knot nematodes, *Meloidogyne* spp., generally are the economically most important nematode pests that parasitise agri- and horticultural crops in South Africa. The aims of the study were to i) identify *Meloidogyne* spp. individuals that infect roots/tubers/pods of crop plants received for diagnostic analyses and from research sites across South Africa using molecular and morphological identification techniques and ii) determine the reproductive potential of *Meloidogyne* species populations identified during the study in a greenhouse experiment (randomised complete block design with six replicates). Deoxyribonucleic acid (DNA) was extracted from mature females obtained from infected, below ground parts of crops and subjected to polymerase chain reaction (PCR) analyses. For the reproduction-potential study, 1000 eggs and second-stage juveniles (J2) of the respective *Meloidogyne* spp. populations identified were inoculated on roots of a susceptible tomato cultivar Floradade. Nematode parameters assessed 56 days later included egg-laying female indices, egg and J2 numbers and reproduction factors/root system. *Meloidogyne incognita* and *M. javanica* proved to be the predominant species that infected maize, potato and soybean crops, while the emerging *M. enterolobii* (= *M. mayaguensis*) have also been identified from pepper and guava roots. Other unknown species have also been detected and are currently being identified. The reproduction potential of the various *Meloidogyne* spp. populations differed substantially within and among species. Positive identification of *M. enterolobii*, which is easily confused with *M. incognita* in terms of its morphological identification will contribute towards research aimed at determining the distribution, life cycle and pathogenicity of this pest. This study is ongoing and knowledge generated will benefit the research fraternity as well as producers and ultimately consumers.

A POTENTIAL BIOCONTROL AND PGPR ACTIVITIES OF BACTERIA *PROVIDENCIA VERMICOL*A AGAINST ROOT KNOT NEMATODE *MELOIDOGYNE JAVANCIA*. **Aish[1,2], Ammar, S.A. Youssef[1], and S.I. Masoud[1].** [1]Agricultural Botany Department, Faculty of Agriculture, Suez Canal University, Ismailia, Egypt; [2]Plant Department, College of Agriculture, University of Baghdad.

A gram negative strain bacteria was isolated from healthy pepper plants grown on naturally infected soil by the plant pathogenic root knot nematode Meloidogyne located at the Agricultural Experimental Farm Station, Faculty of Agriculture, Ismailia. In the dual culture bioassay, an experiment was performed in Petri dishes to examine the effect of the isolated bacteria against egg-masses hatching of the root knot nematode *Meloidogyne javanica*. Results revealed that there was a

pronoun effect of the bacteria on egg-masses hatching under the light microscope. It was observed that the gelatinous matrix turned dark brown to black color. Further examination under the light microscope showed that there were no detected hatching juveniles either motile or immotile. Identification of the bacteria using 16S rRNA proved that the bacteria is *Providencia vermicola* that was reported as a plant growth promoting rhizobacteria (PGPR). In pots experiment under the greenhouse conditions, tomato seedlings inoculated by the dark brown egg-masses failed to exhibit any root galls. The inhibitory effect of bacteria *P. vermicola* against the plant pathogenic nematode *M. javanica* was examined under the greenhouse condition. The obtained results showed that there were no any developed symptoms on tomato roots. In addition, the effect of the isolated bacteria on plant growth parameters was evaluated on tomato seedlings. The obtained results illustrated that the treated tomato seedlings showed a significant increase in the plant growth related parameters, i.e. shoot and root length and fresh and dry weight. This research reports a novel biocontrol and PGPR bacterial activities against the plant pathogenic nematode *M. javanica* that prevents the egg-masses hatching. It is at the best of the authors knowledge, this the first time to demonstrate the effect of the bacteria *P. vermicola* on *M. javanica* egg-masses hatching.

A POTENTIAL BIOCONTROL AND PGPR ACTIVITIES OF BACTERIA *PROVIDENCIA VERMICOL*A AGAINST ROOT KNOT NEMATODE *MELOIDOGYNE JOVANCIA*. **Aish[1,2], Ammar, S.A. Youssef[1], and S.I. Masoud[1].** [1]Agricultural Botany Department, Faculty of Agriculture, Suez Canal University, Ismailia, Egypt; [2]Plant Department, College of Agriculture, University of Baghdad.

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NEMATODE BIODIVERSITY IN SOYBEAN-BASED CROPPING SYSTEMS IN SOUTH AFRICA. **Akhona, M.[1], D. Fourie[1], A. Swart[2], and M. Daneel[3].** [1]North-West University, Unit for Environmental Sciences and Management, Potchefstroom; [2]Agricultural Research Council-Plant Protection Research Institute, Nematology, Pretoria; [3]Agricultural Research Council - Institute for Tropical and Subtropical Crops. Nelspruit.

Soybean (*Glycine max* (L.) Merr.) is an oilseed crop that is continuously expanding in South Africa in terms of its production. However, various nematode pest species parasitise local soybean crops, with *Meloidogyne incognita* and *M. javanica* being the predominant ones. Information on nematode-soybean associations exists locally for conventional soybean crops but not for Round-up Ready® cultivars, which constitute more than 85% of local soybean production. Nematode surveys were thus conducted during 2012 and 2013 growing seasons whereby both soil and root samples were collected at six localities where conventional and Round-up Ready® soybean crops were grown in close proximity. Grass in natural areas adjacent to soybean fields were sampled concurrently to assess the status of both plant-parasitic and non-parasitic nematode assemblages in such ecosystems. Results from this recent research indicated that *Meloidogyne incognita* and *M. javanica* were generally the predominant nematode pests associated with both conventional and Roundup Ready® soybean roots. Root-knot nematode populations ranged from ca. 59 383 eggs and J2/50g roots of Roundup Ready® to ca. 1,225 eggs and J2/50g roots of conventional soybean cultivars during 2012 and from ca. 23,000 eggs and J2/50g roots of Roundup Ready® to ca. 175 000 eggs and J2/50g roots of conventional soybean cultivars during 2013 Interestingly, natural grass hosted up to ca. 1,800 and 1,462 *Meloidogyne* spp. eggs and J2/50g roots during 2012 and 2013, respectively. Other plant-parasitic nematodes that were recorded from root and soil samples from soybean fields and natural vegetation were *Pratylenchus* spp. *Helicotylenchus* spp., *Rotylenchus* spp., *Scutellonema* spp., *Criconemoides* spp., *Criconema* spp., *Tylenchorynchus* spp and Neotylenchidae. In terms of non-parasitic nematodes a variety of fungivores, bacterivores, omnivores, predators as well as entomopathogenic nematodes were identified from soil samples from soybean and natural veld sites. Bacterivores were dominant in terms of their population levels and diversity. This current study found no proof of significant differences in nematode assemblages present in soils where conventional and Roundup Ready® soybean crops are cultivated in South African growing areas.

CHEMICAL ECOLOGY, BEHAVIOR, AND MULTITROPHIC INTERACTIONS OF BENEFICIAL NEMATODES AS A BELOWGROUND INDIRECT PLANT DEFENSE. **Ali, J.G.** Department of Entomology, Michigan State University, East Lansing, MI 48823.

Plant signals play diverse roles to the many organisms that surround them. One facet of this is their ability to manipulate organisms in a manner which protects them or harms herbivores that feed on them. This relationship has more recently been recognized to occur belowground. Here we discuss these belowground interactions, techniques, and findings, focusing on entomopathogenic nematodes and soil nematode chemotaxis in response to plant root cues and potential implications for agroecosystems and fundamental concepts in ecological trophic cascades.

COLONIZATION OF *GLOBODERA PALLIDA*- KILLING FUNGI ON BARLEY ROOTS. **Amiri, Z.M., L.M. Dandurand, and G.R. Knudsen.** Department of Plant, Soil and Entomological Sciences, University of Idaho, Moscow, ID, 83844.

Potato cyst nematodes are internationally regulated pests, which can cause up to 80% yield loss. The pale cyst nematode (PCN), *Globodera pallida,* was found in Idaho in 2006 and placed under a Federal Domestic Quarantine Order (USDA-APHIS) and parallel State Rule (Idaho State Dept. of Agriculture). Eradication efforts have focused on fumigation with methyl bromide, but because of regulatory and environmental constraints, alternatives are urgently needed. Because PCN can survive multiple years as eggs in cysts, requiring presence of a host to hatch, PCN population decline by using crop rotation can take many years. Some nematophagous fungi are known to be natural enemies of cyst-forming nematodes and can be employed as biological agents for pest control. We have shown that both *Plectosphaerella cucumerina*, isolated from infected PCN eggs from Idaho Falls, ID and *Trichoderma harzianum* ThzID1, isolated from soil from Moscow, ID, reduced reproduction of *G. pallida* under greenhouse conditions. Successfully deploying biological control fungi depends on their ability to proliferate and persist under field conditions. We investigated the persistence of *P. cucumerina* and *T. harzianum* ThzID1in PCN- infested fields near Idaho Falls, in 2012 and 2013. Prior to establishing field trial in 2012, the field was fumigated with methyl bromide. Treatments consisted of soil amended with 1) *T. harzianum* at a rate of 80 kg/ha, 2) *P. cucumerina* at a rate of 80 kg/ha, or 3) no amendment. After applying these treatments, barley was planted in all plots. Plots were sampled every 2 weeks over a 10- week period to evaluate rhizosphere colonization. Barley plants were sampled and 1-cm root segments were plated onto selective media for the respective fungi. Rhizosphere soil also was sampled, and estimates of fungal populations were made by dilution assays. Both fungal agents proliferated in the rhizosphere of barley. By July, more than 90% of roots had been colonized by *T. harzianum*, although colonization by *T. harzianum* dropped to non-detectable levels by the end of the experiment. For *P. cucumerina*, the highest level of colonization was found after 6 weeks (45%), but *P. cucumerina* continued to be detected throughout the growing season. These results suggest that these two fungi may both be effective root colonizers under field conditions, but that persistence may differ. Further studies to assess rhizosphere colonization ability of these two fungi are underway.

TRANSCRIPTOMIC ANALYSIS OF THE INSECT *HELIOTHIS VIRESCENS* IN RESPONSE TO THE NEMATODE-BACTERIAL INFECTION. **An, R.[1], K.S. Suri[2], J. Jurat-Fuentes[1], and P.S. Grewal[1].** [1]Department of Entomology, Plant Pathology and Nematology, University of Tennessee, Knoxville, TN 37996; [2]Department of Entomology, Punjab Agricultural University, Ludhiana, Punjab 141004, India.

Entomopathogenic nematodes (EPNs) *Heterorhabditis* and *Steinernema*, and their symbiotic bacteria, *Photorhabdus* and *Xenorhabdus*, respectively are of particular importance in the biological control of insect pests. Investigation of the insect-nematode-bacteria interactions allows deciphering of molecular mechanisms of host defense against parasites and pathogens and this information helps to improve biological pest control potential of these agents. In this study, we used tobacco budworm *Heliothis virescens* as a pest model to dissect host defense mechanisms against nematodes and bacteria through transcriptional analysis of the insect gene expression at specific events during EPN infection. We observed that the EPN *Heterorhabditis bacteriophora* carrying the symbiotic bacteria *Photorhabdus temperate* reached the insect hemocoel 11 h post infection and then released the bacteria into the hemolymph 1 h later. RNA-Seq analyses were performed to profile the differential gene expression of *H. virescens* at 11, 12 and 18 h post-infection with the nematode relative to the untreated control insect. Over 2000 genes were identified to be differentially regulated in the insect after nematode invasion into the hemocoel at 11 h post-infection, of which 2,170 were induced and 292 were repressed. After bacterial release into the hemolymph at 12 h post infection, over 1,500 genes with 1,060 repressed and 522 induced were differentially expressed relative to the previous infection stage. However, only about 500 genes were differentially expressed at 18 h post-infection. Such expression patterns indicate that release of the bacteria into the hemolymph suppressed insect gene expression. Functional annotation of the gene expression data further suggested that the insect immune-related genes were induced upon nematode invasion into the hemocoel but majority of the induced genes were repressed after the bacterial release. These results demonstrate the significance of the partnership between the nematodes and bacteria to establish infections enhancing reproductive success of both organisms.

TOLERANCE TO *BELONOLAIMUS LONGICAUDATUS* IN BERMUDAGRASS (*CYNODON* SPP.). **Aryal, S.[1], W.T. Crow[1], and K.E. Kenworthy[2].** [1]Entomology and Nematology Department; [2]Department of Agronomy, University of Florida, Gainesville, FL 32611.

Research on resistance and tolerance in bermudagrass (*Cynodon* spp.) and other warm-season turfgrasses to sting nematode (*Belonolaimus longicaudatus*) and other plant-parasitic nematodes, has been ongoing at the University of Florida for the past decade. These studies have identified two types of tolerance; i) a cultivar that supports nematode reproduction but suffers minimal root damage from the nematode, and ii) a cultivar that suffers root damage from the nematode but outperforms a standard susceptible cultivar due to vigorous root growth or physical properties. These studies have demonstrated the importance of studying root architecture and growth patterns for understanding nematode susceptibility. Recently, experiments were conducted in microplots and lysimeters to evaluate damage caused by *B. longicaudatus* to turf roots and nitrate leaching, respectively. Both experiments included five genotypes of bermudagrass, including the standard susceptible cultivar 'Tifway', along with two commercial cultivars, 'Celebration' and 'TifSport', and two germplasm lines 'BA132' and 'PI 291590' that were identified as tolerant to sting nematode in previous greenhouse experiments. Root length, surface area, volume, and average diameter were compared between genotypes with and without inoculation with *B. longicaudatus* in the microplots using minirhizotron equipment. Similarly, nitrate leaching was compared between genotypes with and without inoculation with *B. longicaudatus* in the lysimeters. The microplot experiment revealed that TifSport suffered only minimal root loss from *B. longicaudatus* (Type 1 tolerance), and that Celebration and PI 291590 suffered substantial root loss from *B. longicaudatus* but had greater root length, surface area, and volume than the standard cultivar Tifway (Type 2 tolerance). In the lysimeter experiment, among grasses inoculated with *B. longicaudatus* the tolerant germplasms PI 291590 and TifSport had less cumulative nitrate leached than the standard susceptible Tifway. These studies reveal the importance of selection for root growth parameters in breeding programs for nematode tolerance, and inclusion of tolerant cultivars in nematode IPM programs.

EFFECTS OF INFECTION BY *BELONOLAIMUS LONGICAUDATUS* ON NITRATE LEACHING AMONG ST. AUGUSTINEGRASS AND BERMUDAGRASS GENOTYPES. **Aryal S.K.[1], W.T. Crow[1], R. McSorley[1], R.M. Giblin-Davis[1], and K.E. Kenworthy[2].** [1]Entomology and Nematology Department; [2]Agronomy Department, University of Florida, Gainesville, FL 32611.

Nitrate (NO_3^-) leaching in turfgrass ecosystems is of great concern due to the potential to impair groundwater quality. Previous research results indicate that nematode damage to turfgrass roots can increase the potential for nitrate leaching to occur. Turfgrass cultivar selection based on nematode tolerance could reduce the potential NO_3^- leaching. Greenhouse lysimeter studies were conducted in 2013 to 2014 that compared NO_3^- leaching from sting nematode (*Belonolaimus longicaudatus*) susceptible and tolerant genotypes of bermudagrass (*Cynodon* spp.) and St. Augustinegrass (*Stenotaphrum secundatum*), respectively. Five bermudagrass ['Tifway,' standard susceptible; two commercial cultivars ('TifSport' and 'Celebration') and two experimental germplasm ('BA132' and 'PI 291590'), that were identified as tolerant to *B. longicaudatus*] and two St. Augustinegrass ('FX 313', susceptible, and 'Floratam' identified as tolerant to *B. longicaudatus*) genotypes in a 5 x 2 and 2 x 2 factorial design with four replications, respectively. Treatments included were uninoculated control and *B. longicaudatus* inoculated. Total NO_3^- leached from bermudagrass and St. Augustinegrass genotypes were analyzed separately. Mixed models analysis and comparison of LS means indicated increases in NO_3^- leaching under *B. longicaudatus* infested conditions across genotypes of bermudagrass and St. Augustinegrass compared to uninoculated in all leaching events ($P \leq 0.05$). Cumulative NO_3^- leached from *B. longicaudatus* infested turf was less from the tolerant bermudagrass genotypes PI 291590 and TifSport, and the tolerant Floratam St. Augustinegrass than from their susceptible counterparts ($P \leq 0.1$). Therefore, use of nematode tolerant turfgrass genotypes has potential for reducing risk of groundwater contamination from NO_3^-.

SURVEY AND MOLECULAR DIAGNOSTICS OF ROOT-KNOT NEMATODES (*MELOIDOGYNE* SPP.) ON CUT FOLIAGE CROPS FLORIDA. **Baidoo, R.[1], T.M. Mengistu[1], J.A. Brito[2], S. Joseph[1], and W.T. Crow[1].** [1]Entomology and Nematology Department, University of Florida, PO Box 110620, Natural Area, Dr., Gainesville, FL 32611; [2]Florida Division of Plant Industry, 1119 SW 34th St., Gainesville, FL 32608.

Florida is the hub of cut foliage business in the USA and accounts for more than 75% of the national cut foliage production. Unfortunately, root-knot nematodes (*Meloidogyne* spp.) are a serious problem on these crops, rendering many farms unproductive. Currently, information on *Meloidogyne* spp. occurring on most commonly cultivated cut foliage crops in Florida and tools for their rapid identification is lacking. Therefore, a survey was conducted at the University of Florida to identify specific root-knot nematodes infecting common ornamental cut foliage plants in Florida; and to identify molecular markers for rapid detection and identification of these nematodes. A total of 200 root samples were collected from four cut foliage plant species *(Pittosporum tobira*, *Liriope gigantea*, *Ruscus hypophyllum*, and *Aspidistra elatior*) growing in five farms in Florida. A total of 10 *Meloidogyne* female samples were collected per plant species per farm and genomic DNA extracted individually using the NaOH method. The mitochondrial DNA region between the cytochrome oxidase II subunit and large

ribosomal RNA was amplified from the genomic DNA using TRNAH/MRH106 and MORF/MTHIS primer sets. The resultant PCR product for TRNAH/MRH106 was subjected to *Mnl*I and *Hinf* I endonuclease digestion to discriminate species. Species-specific primers were used to further verify the identity of the nematodes involved. The *Meloidogyne* spp. identified include *M. incognita*, *M. javanica*, *M. hapla*, *M. hispanica* and an unknown *Meloidogyn*e spp. *Meloidogyne incognita* was the dominant *Meloidogyne* spp. constituting more than 60% of the total number of *Meloidogyne* spp. identified followed by *M. javanica*. Sequences of mtDNA amplified by TRNAH/MRH106 primer set from a single female of *M. hispanica* and *M. hapla*, respectively aligned with mtDNA sequence of *M. hispanica* (Accession number JN673274) and *M. hapla* (Accession # L76262) retrieved from GenBank with 99% similarity. *Meloidogyne hispanica* has never been reported in Florida, although the incidence of both *M. hispanica* on *P. tobira* and *M. hapla* on *R. hypophyllum* in Florida was less than 1%. PCR amplification of the mtDNA gene followed by endonuclease digestion by *Mnl*I and *Hinf* I was useful and reliable tool in discriminating the *Meloidogyne* spp.

SPATIAL ECOLOGY OF ENTOMOPATHOGENIC NEMATODES IN SOIL. **Bal, H.K.[1] and P.S. Grewal[1,2].** [1]Department of Entomology, The Ohio State University, OARDC, Wooster, Ohio 44691; [2]Department of Entomology and Plant Pathology, University of Tennessee, Knoxville, Tennessee 37996.

Laboratory studies have identified a dichotomy in foraging behavior of entomopathogenic nematodes (EPNs) but little is known about their dispersal patterns in the field. We assessed the rate of active lateral movement and dispersal patterns of the two EPN species with contrasting foraging strategies, cruiser, *Heterorhabditis bacteriophora* and ambusher, *Steinernema carpocapsae* at the population level from nematode-infected host cadavers in soil in the absence of hosts, in the presence of vegetation and mobile and non-mobile hosts in the greenhouse conditions and further, quantified their short-term dispersal potential in the field and finally, genetically selected ambusher, *S. carpocapsae* for enhanced dispersal. The results revealed that the two species differed in the spatio-temporal pattern of dispersal but showed similar average population displacement (~6 cm/day) in the absence of hosts. A greater percentage of *S. carpocapsae* (4%) dispersed faster than the fastest *H. bacteriophora* (2%) to larger distances, 30-61 cm. These apparent 'sprinters' may represent an adaptive dispersal strategy by the otherwise ambush forager *S. carpocapsae* in the absence of hosts. *S. carpocapsae* showed positive response to selection for enhanced dispersal with associated trade-offs including reduced reproduction capacity and nictation ability. The farthest reaching infective juveniles (IJs) of the selected lines comprised more males (72%) than the foundation population (44%). Vegetation enhanced dispersal of both species but more so for *H. bacteriophora*. Their dispersal behavior was affected by both the presence and absence of hosts and by their mobility. Mobile hosts enhanced dispersal of both species. *S. carpocapsae* also had higher average population displacement than *H. bacteriophora* in the presence of both, non-mobile and mobile hosts. A large proportion of IJs of both species stayed near (≤ 3.8 cm) the source cadaver (88-96% *S. carpocapsae*; 67–79% *H. bacteriophora*), and the proportion of IJs reaching the farthest distance (11.4 cm) was significantly higher for *S. carpocapsae* (1.4%) than *H. bacteriophora* (0.4%) in the presence of mobile hosts. In the field, both the species showed equivalent potential to disperse up to 2 m, actively or passively, in both grass and cultivated potatoes, with equal arthropod abundance in the two habitats over a period of four days. While *H. bacteriophora* was associated with mites, *S. carpocapsae* was detected in larger numbers in pitfall traps, likely entering the traps on various arthropods. Spatial distributions of the two EPN species after dispersing from a grassy border into the adjacent cultivated field plots were more uniform for *S. carpocapsae* than for *H. bacteriophora*, based on Moran's I, Geary's c and spatial analysis by distance indices (SADIE). The results provide quantitative understanding of EPN dispersal and suggest genetic selection as a promising approach for enhancing *S. carpocapsae* dispersal, which would have implications for designing strategies for innundative application and establishing sustainable populations of these important biocontrol agents.

EFFECT OF SOIL MANAGEMENT ON SOIL FOOD WEB IN LONG-TERM ORGANIC AND TRANSITIONING FARMING SYSTEMS. **Bal, H.K.[1], K.R. Islam[2], E.L. McCoy[3], S. Kumarappan[4], A. Sundermeier[5], and P.S. Grewal[1,6].** [1]Department of Entomology, OARDC, Ohio State University, Wooster, OH 44691; [2]OSU South Centers, Piketon, OH 45661; [3]School of Environment and Natural Resources, OARDC, Ohio State University, Wooster, OH 44691; [4]Agricultural Technical Institute, Wooster, OH 44691; [5]Ohio State University Extension, Wood County, Bowling Green, OH 43402; [6]Current address: Department of Entomology and Plant Pathology, University of Tennessee, Knoxville, TN 37996.

Sustainable management practices enhance ecosystem services of organic production systems provided by the soil food web diversity. We hypothesized that a combination of continuous no-till (NT), multi-functional cover crops, and natural amendments (mined Zeolite) would produce greater ecosystem services by increasing soil food web structural and functional complexity in long-term organic systems. We compared 2 levels of tillage (conventional till, CT and NT), 3 levels of zeolite (0, 50 100 kg/ha) and 3 crop phases (corn-soybean-spelt) at three locations, long-term organic farms at Bowling Green and West Salem, and transitioning experiments at Piketon, Ohio to evaluate the soil food web (represented by nematode community). Multivariate repeated measures analysis of variance showed statistically significant differences between the three sites in the pattern of abundance of nematodes belonging to different feeding types and cp values as well as in different indicator indices over a period of summer 2013 through fall 2014. Nematode-faunal analysis showed that the soil food web in

the long-term organic farms was significantly enriched (Enrichment Index, EI: 61%), structured (Structure Index, SI: 47%) and matured (Maturity Index, MI: 2.2%) than in transitioning systems (EI: 51%; SI: 33%; MI: 2%), with greater bacterial decomposition channels (Channel Index, CI <50%) in all three sites at most sampling times. The soil food web became more enriched and structured in NT than in CT over time with significant improvement in the structure index in fall 2014. While there was a significant interaction in all the parameters, site, tillage, crop phases, zeolite in structure, maturity and combined maturity indices and in cp1 and cp3 nematodes, higher trophic groups (cp4 and cp5) showed a significant interaction between site, tillage and zeolite treatments over time. Depth of soil sampling had a significant impact on the pattern of abundance of omnivorous, cp3 and cp4 nematodes, and enrichment, structure and plant parasitic indices over time. Specifically, significantly greater number of all the nematodes (based on both feeding types and cp values) were found in the top (0-2'') than the bottom (2-8'') portion of the soil; however most of the indicator indices (EI, PPI, CMI) were significantly higher in the bottom (2-8'') than the top (0-2'') portion of the soil at most time points. Long-term study is on-going to further validate enhanced soil food web structural and functional complexity in long-term organic farming systems managed with the proposed soil management practices.

HOST STATUS OF A NEW *MELOIDOGYNE* SPECIES FOUND PARASITIZING YELLOW AND PURPLE NUTSEDGES. **Beacham, J.[1], S. Thomas[1], J. Schroeder[2], L. Holland[3], E. Morris[1], N. Schmidt[4], L. Murray[5], F. Solano-Campos[6], S. Hanson[1], and J.D. Eisenback[7].** [1]Department of Entomology, Plant Pathology and Weed Science, P.O. Box 30003 MSC 3BE, New Mexico State University, Las Cruces, NM 88003; [2]USDA Office of Pest Management Policy, 1400 Independence Avenue, Washington DC; [3]Dept. of Plant Pathology, Washington State University, Pullman; [4]Economics, Applied Statistic and International Business Department, New Mexico State University, Las Cruces; [5]Department of Statistics, Kansas State University, Manhattan; [6]Escuela de Ciencias Biológicas, Universidad Nacional, Costa Rica; [7]Department of Plant Pathology, Virginia Tech, Blacksburg.

Replicated greenhouse studies were conducted to assess host range of a new *Meloidogyne* species (hereafter referred to as NSRKN) discovered when harvesting a tomato bioassay of tubers from cultures of yellow nutsedge (YNS, *Cyperus esculentus*) and purple nutsedge (PNS, *Cyperus rotundus*). In this bioassay, prominent galling was observed on PNS roots but was absent on the associated 'Rutgers' tomato roots. Dissection of these root galls revealed small *Meloidogyne* females with egg masses primarily contained inside the root tissue.

Alfalfa, chile pepper, corn, cotton, onion and sorghum (all common New Mexico field crops), barley, oats, perennial ryegrass, wheat, winter rye, and bentgrass, along with tomato, YNS and PNS as negative and positive controls were each inoculated with 2,000 NSRKN eggs. After ~750 cumulative heat units above 10C, individual plants were harvested, washed roots were blender-macerated in a 1.0% NaOCl solution and rinsed over nested sieves onto a final 400-mesh sieve for collection and quantification of egg production. Galling was observed and eggs were recovered in variable quantities from YNS, PNS, barley, bentgrass, perennial ryegrass, wheat, oats, winter rye, alfalfa and sorghum. Chile pepper, corn, cotton, and onion were non-hosts as no eggs were recovered (n=20) from these annual host plants. A gamma distribution was used to calculate and compare LSMEANS of Reproductive Factors (RF). YNS was the best host overall with 5-10x the RF value (28.3) compared to PNS (5.5), barley (2.9) and bentgrass (2.3). Both wheat (.77) and ryegrass (.89) were poor hosts.

Concurrent with the greenhouse studies, DNA was extracted from 56 individually isolated NSRKN second stage juveniles (J2) obtained from multiple infested nutsedge sources and sequence of the ~550bp fragment between the mitochondrial cytochrome oxidase subunit II gene (COII) and the 16S rRNA gene (Powers & Harris, 1993, JON 25:1-6) showed all J2 had high similarity to each other, but only 90% similarity to *M. graminicola* with even less similarity to *M. naasi* in databases containing *Meloidogyne* mitochondrial sequences. Taxonomic characterization is currently underway. Preliminary results indicate that the female stylet is unique for the genus as the posterior of the knobs are composed of several angular edges; J2 have relatively long, thin tails; and perineal patterns most closely resemble those of *M. naasi*. Though not an apparent threat to NM annual crops, further study is needed to evaluate pathogenicity of this new species on gramineacious hosts.

KLEPTOPARASITIC RHABDITID AND DIPLOGASTRID NEMATODES (RHABDITIDA) IN THE INTESTINE OF MILLIPEDS (DIPLOPODA). **Bernard, E.C. and G. Phillips.** Entomology & Plant Pathology, University of Tennessee, 2505 E. J. Chapman Drive, 370 Plant Biotechnology, Knoxville, TN 37996-4560.

Larger millipeds typically support significant numbers of rhigonematid and thelastomatid nematodes in their intestines. These nematodes are specialized for this internal habitat as obligate kleptoparasites, consuming bacteria and minute bits of comminuted organic matter ingested by the milliped, apparently without harmful effect on the host. We are now intensively investigating these nematodes and their host-parasite relationships. Among more than 500 dissections we have found *Diplogasteroides* sp. several times and an unplaced species of Rhabditidae *s. str.* once. All stages of the *Diplogasteroides* sp. were collected from the mid-intestines of Florida *Narceus gordanus* (Spirobolidae) and Tennessee *Pachydesmus* sp. (Xystodesmidae), and successfully cultured on quarter-strength cornmeal agar (CMA), with bacteria derived from the intestine as the food source. This amphimictic, non-nictating species is distinct from described *Diplogasteroides* in having a file of three slender teeth on one side of the metastom and in having much smaller, stouter spicules. These collections

comprise the first report of a diplogastrid as an internal milliped parasite. In culture these nematodes apparently are attracted to each other by a pheromone, as scattered individuals in an agar dish soon congregate in one area. The rhabditid was collected as several hundred nictating dauer juveniles from the posterior intestine of *N. gordanus*. Dauers placed on CMA matured within two days to egg-laying hermaphrodites. Males have not been observed in this isolate. Culture of this rhabditid initially was complicated by the presence of the nematophagous fungus *Drechmeria coniospora*, which also must have originated from the milliped intestine. Most reports of Rhabditidae in millipeds are for *Oscheius necromenus*, which has been proposed as a biological control agent against pest millipeds, especially in Australia. This nematode presumably transports bacteria on its cuticle into the milliped, which then proliferate and kill it. The presence of several hundred dauers in *N. gordanus* suggests that this rhabditid could have an advanced compatible relationship with its host. Normal bacterial levels in the milliped gut undoubtedly are sufficient for rhabditid development, since the much larger obligate kleptoparasites also subsist largely on bacteria. Another possibility is that bacteria pathogenic to millipeds do not exist in temperate North America.

DOES ROOT-KNOT NEMATODE (*MELOIDOGYNE INCOGNITA*) ON TOMATO (*SOLANUM LYCOPERSICUM*) AFFECT DECADAL TRENDS IN THE EL NIÑO SOUTHERN OSCILLATION (ENSO)? **Bird, E.E.[1], I.D. Claire[2], P. Brain[3], R.M. Pitt[4], M.T. Head[5], I.M Boring[6], and B.A. Ware[7].** [1]Dept. of Plant Psychology, Elroy Jetson Space Magic Institute, Houston, TX 77005; [2]Dept. of Advanced Advances, Lawrence of Arabia National Lab, Liverwurst, CA 94550-9234; [3]Phytoneural Anatomy Dept., Steve Austin State University, Nachotacos, TX 75962; [4]Institute for the Advanced Study of Plant and Animal Interactions, Carnegie Cucumber University, Spittsburg, PA 15213; [5]Center for Phytoengenuity, Smirkell College, Smirkell, IA 50112; [6]Dept. of Teleology and Orthogenesis, Kryptonne National Labs, Lamont-Sanford, IL 60439.

As best we can discern, root-knot on tomato has no effect on decadal trends in the El Niño Southern Oscillation (ENSO).

ROLES OF BIOLOGICAL PROTECTION SOLUTIONS IN 21ST CENTURY CROP PRODUCTION SYSTEMS. **Bird, G.W.** Department of Entomology, Michigan State University, East Lansing, MI 48842.

In a world of 7.3 billion, a significant portion of temperate, tropical and desert biomes have been modified for food production. The hypotheses for this presentation are: 1) these changes resulted in systems that are very favorable for risk to soil-borne pests/pathogens and 2) biological solutions have significant potential for managing these organisms in 21st Century crop production systems. The solutions consist of directly lowering soil-borne pest/pathogen population densities through use of biological control agents or modifying soil ecosystems in ways that enhance biological diversity/soil health. Various biological control products consisting of bacteria, fungi, nematodes or trap/bio-fumigant plant cultivars have been developed and marketed for control of soil-borne pests/pathogens. Challenges associated with use of these materials include, application technology, target organism specificity, storage limitations and virulence retention. Current technologies for risk reduction and biological diversity enhancement through habitat modification include cover crops, compost/organic amendments, reduced tillage and inclusion of animals in the system. Crops not currently in a farming system may be necessary to provide new windows for optimal use of biological protection solutions, including simulating positive attributes of the site's native biome. Some of the most progressive large farms in the U.S. are currently including biological protection solutions in their research related to transitioning to a new system of bio-based agriculture.

ENVIRONMENTAL DRIVERS OF TRAIT CHANGES IN *PHOTORHABDUS LUMINESCENS*. **Blackburn, D.[1], B. Crawford[1], D.I. Shapiro-Ilan[2], and B.J. Adams[1].** [1]Biology Department, Brigham Young University, Provo, UT 84602; [2]USDA-ARS, Southeastern Fruit and Tree Nut Research Laboratory, Byron, GA 31008.

Biological control agents have become increasingly important in integrated pest management programs. However, certain traits of these agents that are needed for efficient biocontrol often decrease or are lost during *in vitro* rearing. Trait deterioration can result from genetic or environmental causes (such as nutrition). Entomopathogenic nematodes (EPNs) are biocontrol agents that kill their insect targets with the help of a symbiotic bacterium. EPNs and their bacterial symbionts often exhibit trait deterioration when reared under laboratory conditions. EPN trait deterioration has been attributed (at least in part) to genetic causes; however, the underlying causes of trait deterioration in the bacterial endosymbiont have not been explored. In this study the EPN symbiont *Photorhabdus luminescens* was monitored for the deterioration of three traits; inclusion body production, reproductive potential, and virulence, in three different nutritional environments; lipid liquid medium (LLM), nutrient broth (NB), and tryptic soy broth+yeast extract (TSY). Significant trait deterioration did not occur for any of the traits in any environment. However, there was an increase in inclusion body production in TSY. Additionally, there were differences in growth rates within NB and TSY sub-cultured population lines and one TSY sub-population line was less virulent than the other two. However, returning bacteria to LLM restored all traits to wild-type levels. We infer the observed trait deterioration in *Photorhabdus* was minimal, and the deterioration that was observed appeared to be driven by environmental conditions as opposed to

stable genetic changes. Our data suggest that variation among important biological control traits of *in vitro* cultures of *Photorhabdus luminescens* is more likely due to environmental variation than inadvertent laboratory selection or other genetic processes.

MELOIDOGYNE PARTITYLA INFECTING WATER OAK (*QUERCUS NIGRA*) IN FLORIDA, USA. **Brito, J.A.,[1], T. Smith[1], M.F. Achinelly[2], T.S.A. Monteiro[3], and D.W. Dickson[4].** [1]Florida Department of Agriculture and Consumer Services, Division of Plant Industry, Gainesville, FL 32608; [2]University of La Plata, La Plata,1900, Argentina; [3]Department of Phytopathology, University of Vicosa, Vicosa, MG, 36570-900, Brazil; [4]Department of Entomology and Nematology, University of Florida, Gainesville, FL 32611.

The pecan root-knot nematode *M. partityla* was first reported infecting pecan seedlings in a nursery in 2005 and laurel oak (*Quercus laurifolia*) in 2009 in Florida. The infection of laurel oak was the first report of a plant host outside of the Junglandaceae family. In January 2009 and February 2015, roots of water oak (*Quercus nigra*) exhibiting large coalesced galls and egg masses resembling those induced by root-knot nematodes were found in two different home gardens, Alachua Co., FL. Second-stage juveniles, female and male root-knot nematodes extracted from the oak roots were identified based on their morphology. In addition, females were subjected to polyacrylamide gel electrophoresis to identify their isozyme phenotypes. Key morphological features used were the perineal pattern of females, the swollen and clear longitudinal grooves in rectum of J2, and thickened region between the stylet cone and stylet shaft of males. The isozyme phenotypes (EST=Mp3; MDH=N1a) were consistent with that previously reported for *M. partityla*. Identification was further confirmed using molecular analyses of the mitochondrial DNA (mtDNA) region between CO II and 16S; 18S rDNA; and rDNA ITS, a region containing ITS1, 5.8S and part of ITS2. DNA extracted from *M. partityla* infecting pecan (*Carya illinoinensis*) was used as a control. mtDNA region was amplified with the C2F3/1108 primer set and produced a fragment of approximately 530 bp, whereas the 8S rDNA amplified using primers 18Ss1.2 (5'-GGCGATCAGATACCGCCCTAGTT-3') and 18SR2B (5'-TACAAAGGGCAGGGACGTAAT-3') resulted in an amplicon of ca 630 bp, both of which are identical to those previously reported for *M. partityla*. The rDNA ITS was amplified with ITS-1 F (CGCAGTGGCTTGAACCGG) and a primer shown only to anneal in *M. partityla*, MpSpec (TGAACTTTTATTGGTGAAAG) and sequenced. The amplification size using this species-specific primer combination for females found infecting both water oak (GenBank # KR047556) and pecan (GenBank # KR047555) was ca. 500 bp, which is in agreement with that found in *M. partityla* infecting pecan in Arizona, Florida, Georgia, New Mexico, Oklahoma and Texas. Studies are in progress to determine whether *M. partityla* infecting oaks can also infect pecan and vice versa, and further elucidate the phylogenetic relationship among these nematode populations.

MELOIDOGYNE PARTITYLA INFECTING WATER OAK (*QUERCUS NIGRA*) IN FLORIDA, USA. **Brito, J.A[1], T. Smith[1], M.F. Achinelly[2], T.S.A. Monteiro[3], and D.W. Dickson[4].** [1]Florida Department of Agriculture and Consumer Services, Division of Plant Industry, Gainesville, FL 32608; [2]University of La Plata, La Plata,1900, Argentina; [3]Department of Phytopathology, University of Vicosa, Vicosa, MG, 36570-900, Brazil; [4]Department of Entomology and Nematology, University of Florida, Gainesville, FL 32611.

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REPRODUCTION OF *MELOIDOGYNE FLORIDENSIS* ON SELECTED ROOT-KNOT NEMATODE RESISTANT PLANT SPECIES. **Brito, J.A.[1], S.J.S. Vau[2], and D.W. Dickson[2].** [1]Florida Department of Agriculture and Consumer Services, Division of Plant Industry, Gainesville, FL 32608; [2]Department of Entomology and Nematology, University of Florida, Gainesville, FL 32611.

The peach root-knot nematode, *Meloidogyne floridensis*, was first reported in 2004. The species, which is known to occur only in Florida, is reported to be able to infect and reproduce on root-knot nematode resistante peach rootstocks 'Flordaguard', 'Nemaguard', 'Nemared' and 'Okinawa'. Our objective was to determine the reproductive capability of *M. floridensis* on three *Mi-1* gene tomato cvs. Amelia, Crista and Fletcher, cowpea cvs. Iron Clay (resistant, *Rk* gene) and California 05 (susceptible), and peanut cvs. Tifguard (resistant) and Florida 07 (susceptible). Tomato cv. Agriset 334 was used as a susceptible control. Seedlings were grown in clay pots (15-cm-diam.), inoculated with 5,000 eggs or second-stage juveniles/seedlings, and kept in a greenhouse for 60 days. At harvest, root systems were washed and stained with food coloring for 20 minutes. Root galling and egg mass indicies (0-5 scale), reproductive factor (Rf), and number of eggs/g fresh roots (g fr) were determined. Root galling ranged from 1.5 on Iron Clay cowpea to 5.0 on Amelia, Crista, Flecher and Agriset 334 tomato, whereas egg mass indices ranged from 1.8 on Iron Clay cowpea to 5.0 on Amelia and Agriset 334 tomato. The Rf value for Agriset 334 tomato (control) was 82.9 with 10,676 eggs/g fr. The number of eggs/g fr and reproductive factor on *Mi-1* gene tomato were 2, 348 (Rf=12.4); 3,184 (13.2) and 1,074 (5.4) for Amelia, Crista and Fletcher, respectively. Likewise, this nematode also reproduced well on *Rk* gene Iron Clay (618 eggs/g fr; RF=2.8) and California 05 (1,244 egg/g fr; Rf=6.7). In summary, *M. floridensis* was able to infect and reproduce on all plant species except for the two peanut cultivars.

GENOME SKIMMING: NOVEL INSIGHTS INTO PLANT-PARASITIC NEMATODE GENOMES AND POTENTIAL AVENUES FOR MANAGEMENT. **Brown, A.M.V.[1], D.K. Howe[1], A.B. Peetz[2], W.S. Phillips[2], I.A. Zasada[2], and D.R. Denver[1].** [1]Department of Integrative Biology, 3029 Cordley Hall, Oregon State University, Corvallis, OR 97331, [2]USDA-ARS Horticultural Crops Research Laboratory, 3420 NW Orchard Avenue, Corvallis, OR 97330.

In exploring ways to bridge the gaps between classical nematology, genomics, and applied management research, we have developed a simple, but fruitful bioinformatics pipeline for analyzing genomic data. The genomic approach is analogous to skimming, meaning taking the richest part at the surface, as for cream on milk. It has the advantage of inexpensively yielding important insights that can be explored with further directed experiments. We began with shallow (low coverage) multiplex sequencing from bulk nematode samples, using medium-length reads and inserts (Illumina MiSeq PE 301 bp reads; ~700 bp inserts). In a short time (~2 weeks), we obtained results, which, with brief analysis using free software on a single computer, produced six new nematode draft genomes at ~8-30X coverage each, from a single MiSeq run (~$2,000). The two keys to this success were the relatively small size (~100 Mbp) of nematode genomes, and the increased length of the reads and inserts, compared with shorter reads from state-of-the-art sequencing of just a few years ago. Even without the very labor-intensive genome-finishing and annotation steps, our results revealed practically important genome content, population genetic, and community differences of the six plant-parasitic nematodes, *Anguina agrostis, Globodera ellingtonae, Pratylenchus neglectus, P. penetrans, P. thornei,* and *Xiphinema americanum*. For example, we estimated genome size, %GC, and effector gene differences of these nematodes. Several bacterial endosymbionts were identified including *Xiphinematobacter*, *Wolbachia,* and *Cardinium*, and other possible associates like *Pseudomonas fluorescens* and *Janthinobacterium*. The discovery of new plant-parasitic nematode symbionts offers a novel potential avenue for management. Additionally, the bulk samples revealed different mixtures of strains and suggested possible complex ploidy in some of the analyzed Tylenchids. We propose a wider use of this approach to yield broad insights into plant-parasitic nematode basic biology, evolutionary relationships, and prospective targets for management.

MANAGEMENT OF PLANT PARASITIC NEMATODES IN CALIFORNIA GRAPE PRODUCTION WITH SPIROTETRAMAT. **Cabrera, J.A.[1] and J. Bell[2].** [1]Bayer CropScience, 266 South Monroe Avenue, Fresno, CA 93706; [2]Bayer CropScience, 2 TW Alexander Drive, Research Triangle Park, NC 27709.

Most grape varieties and rootstocks of table, raisin and wine grapes in California are susceptible to multiple nematodes species, such as root-knot (*Meloidogyne* spp.), citrus (*Tylenchulus semipeneterans*), ring (*Mesocriconema* spp.), and dagger (*Xiphinema* spp.) nematodes. In the last decades, methyl bromide was used to control plant parasitic nematodes, however, this fumigant has been phased out under the provisions of the U.S. Clean Air Act and the international Montreal Protocol Treaty because it is an ozone depleting molecule. Other fumigants are highly regulated and can have township caps and buffer zone requirements. Certain available grape rootstocks have tolerance/resistance to some nematodes, but not all. In addition, certain nematode populations can increase in the tolerant/resistant rootstocks over time. Therefore, alternative strategies to manage nematodes are required. Spirotetramat is a xylem and phloem mobile insecticide which has shown activity against plant parasitic nematodes in grape vines. The spirotetramat-enol form that is formed inside the plant has shown to reduce plant parasitic nematode populations in soil. In Fresno County, spirotetramat applied at 6.25 or 12.5 fl. oz. per acre with 0.25% of a non-ionic surfactant in 75 gallons of water per acre, reduced the number of root-knot, citrus, and ring

nematodes. In another vineyard in Kern County, spirotetramat applied at 6.25 fl. oz. per acre reduced the number of ring nematodes and increased grape yield compared to the untreated control. In conclusion, spirotetramat showed effective activity for the management of plant parasitic nematodes in grape production.

PERFORMANCE OF FLUOPYRAM AGAINST ROOT-KNOT NEMATODES APPLIED THROUGH CHEMIGATION AT DIFFERENT TIMINGS ON TOMATO IN CALIFORNIA. **Cabrera, J.A.[1], A. Kurokawa[1], and S. Krueger[2].** [1]Bayer CropScience, 266 South Monroe Avenue, Fresno, CA 93706; [2]Bayer CropScience, 2 TW Alexander Drive, Research Triangle Park, NC 27709.

Root-knot nematodes (*Meloidogyne* spp.) are of the most economically important nematodes in vegetable production worldwide often causing root galling and yield decline, in particular in California tomato production systems. A novel chemistry that has shown nematicide activity is fluopyram. Fluopyram is a succinate dehydrogenase/succinate-coenzyme Q reductase inhibitor which is a new nematicide mode of action. The objective of this investigation was to evaluate the performance of fluopyram applied through chemigation at different timings against root-knot nematodes on tomatoes in California field conditions. Two repeated field trials revealed that fluopyram chemigated before transplanting followed by an at-transplanting application was significantly more effective controlling root-knot nematodes compared to a conventional oxamyl program. At transplanting chemigation with fluopyram followed by a second application 2 weeks later provided high nematode control activity throughout the tomato growing season. Replacing the first oxamyl application with fluopyram in an oxamyl chemigation program provided higher nematode control than using oxamyl alone. In conclusion, the efficacy of fluopyram against root-knot nematodes was higher than oxamyl in all treatments evaluated, providing adequate root-knot nematode control under field conditions. These studies showed that fluopyram can be an effective option to manage root-knot nematodes in tomato production. Note: fluopyram is not yet registered for sale or use against nematodes in California.

ZINC PYRITHIONE IS NEMATICIDAL; EVIDENCE FOR A ROLE FOR KQT POTASSIUM CHANNELS. **Calahorro, F.[1], Gafar, H.[2], X. König[2], S. Böhm[2], K. Sullivan[1], V. O'Connor[1], H. Morgan[1], and L. Holden-Dye[1].** [1]University of Southampton, Institute for Life Sciences, Highfield Campus, Southampton SO17 1BJ, UK; [2]Department of Neurophysiology and Neuropharmacology, Centre of Physiology and Pharmacology, Medical University Vienna, Schwarzspanierstraße 17, 1090 Vienna. Austria.

Zinc pyrithione (ZP) is an antimicrobial and antifungal compound which is the active constituent of anti-dandruff shampoo. We have found that ZP causes larval arrest in *Caenorhabditis elegans*, an effect that is apparently independent of any antimicrobial action on the bacterial lawn on which the worms feed. Here we report on ZP's mode of action. Intriguingly ZP has previously been reported to interact with mammalian voltage-gated potassium channels (Xiong *et al*., 2007) and therefore we addressed whether or not this might explain the biological activity of ZP against *C. elegan*s. Through heterologous expression in *Xenopus* oocytes we showed that ZP is an activator of KQT-1, a *C. elegans* orthologue of the mammalian KCNQ potassium channels. In behavioural studies ZP impacts on *C. elegans* chemotaxis and feeding behaviour. These effects are phenocopied to some extent in a *C. elegans kqt-1* mutant which is defective in pharyngeal pumping. Moreover, these mutants present a defective adaptation response when they are food exposed and/or deprived. *kqt-1* fails to recover the normal pharyngeal pumping through the transitions between food/non-food/food, indicating a possible interruption within the pharyngeal neuronal microcircuits. A subset of the actions of ZP are occluded in the kqt-1 mutant suggesting a role for KQT-1 in mediating these effects on behaviour. The effects on the pharyngeal system were investigated in more detail using electrophysiological characterisation with NeuroChip (Hu *et al*., 2013). This showed that the frequency of pharyngeal pumping in wild-type nematodes was altered in the presence of lower concentrations of ZP. Treated animals showed a lower frequency of EPGs (ElectroPharyngeoGram), a useful readout of the electrophysiological signals from the *C. elegans* pharynx, indicating that ZP is able to inhibit the normal pharyngeal activity in *C. elegans*. The expression pattern of *kqt-1* in the worm, in mechanosensory neurones and in pharyngeal muscle is consistent with ZP acting on KQT-1 to decrease pharyngeal excitability and inhibit pharyngeal pumping.

Overall, these data suggest that the antimicrobial actions of ZP translate to nematicidal action through activation of KQT-1, as well as a role impacting on the pharyngeal microcircuit, inhibiting in this way the physiological activity of the nematode *C. elegans*. This has implications for the discovery of new nematicidal targets and may also have relevance to the ecotoxicity of ZP, a widely used antimicrobial agent.

NEUROCHIP: A MICROFLUIDIC DEVICE FOR IMPROVED RESOLUTION AND THROUGHPUT FOR CHEMICAL SCREENING FOR CROP PROTECTION AND ANIMAL HEALTH. **Calahorro, F., T. Fereiro, H. Morgan, V. O'Connor, and L. Holden-Dye.** Centre for Biological Sciences and Centre for Hybrid Biodevices. Blg 85. Highfield Campus. University of Southampton. SO17 1BJ, UK.

Caenorhabditis elegans pharynx provides an excellent systems level platform to investigate the biological activity of chemicals with unknown mode of action. Its rhythmic cycle of contraction and relaxation is regulated by a microcircuit which harbours a diverse array of signalling molecules including known anthelmintic and nematicidal targets. We have previously

described an integrated microfluidic and electrophysiological experimental platform that records a waveform reporting the activity of the pharyngeal circuitry (Hu *et al*., 2013) and its adaption for measuring oesophageal-stylet function in plant parasitic nematodes (Hu *et al*., 2014). Here we report the application of this approach for providing phenotypic signatures for different classes of neuroactive and nematicidal chemicals. We have optimized medium throughput plate assays in which worms can be cultivated in chemicals of interest overnight prior to investigation in the device. This approach is particularly useful in instances where access across the cuticle is slow or mode of action implies that chronic exposure is required for biological activity. The format and ergonomics of loading are suited to investigation of both the chronic or acute effects of important classes of chemicals on wild-type, mutant or genetically modified *C. elegans* sculpted to express targets of experimentally intractable nematode species

UNDERSTANDING THE ASSEMBLAGE OF ENTOMOPATHOGENIC NEMATODES SOIL FOOD WEB IN NATURAL AND AGRICULTURAL AREAS: NEW INSIGHTS FROM A SWISS ECOLOGICAL RESEARCH NETWORK. **Campos-Herrera, R.[1], G. Jaffuel[1], X. Chiriboga[1], R. Blanco-Pérez[1], A.M. Fesselet[2], S. Hug[3], R.G. Meuli[3], P. Mäder[4], F. Mascher[2], and T.C.J. Turlings[1].** [1]FARCE Laboratory, University of Neuchâtel, CH-2000 Neuchâtel (Switzerland); [2]DEFR, Agroscope, Institut des Sciences en Production Végétale IPV, CH-1260 Nyon (Switzerland); [3]Agroscope, Institute for Sustainability Sciences ISS, Reckenholz, CH-8046 Zürich (Switzerland); [4]FiBL, Research Institute of Organic Agriculture, CH-5070 Frick (Switzerland).

Entomopathogenic nematodes (EPNs) are excellent biological control agents of numerous soil dwelling insect pests. They occur in both natural and agricultural ecosystems worldwide. As part of a research consortium that explores how soil health can be improved by applying ecologically sound and rational approaches (NRP 68: Biology), we study how EPNs can be better exploited for biological control in annual crops. Also, we aim to document the EPN assemblage and associated organisms in natural soil food webs. This knowledge will serve to better understand the mechanisms that shape complex soil communities and to identify key factors driving EPN prevalence and activity. We hypothesized that the frequent mechanical disturbance of soils in annual crops will compromise the natural EPN community due to exposure to harsh abiotic conditions and limited host availability. This impact should be reflected in lower EPN presence in agricultural soils as compared to natural areas. These factors are expected to also negatively affect other members of the soil food web. To test this we explored EPN occurrence in Swiss ecosystem by combining traditional methods and state-of-the-art molecular tools. Seven new real-time qPCR protocols for Europe EPN species were developed. EPN activity and occurrence was measured in spring and autumn 2013 in three long term running Swiss field trials, two of our surveys focused on tillage soils (Nyon) and one on the impact of crop type (maize, wheat and grass) and fertilization program (DOK trial, Therwil). The analysis of 280 soil samples revealed very low numbers of EPN in annual agro-ecosystem. Natural enemies followed similar spatial patterns than EPN. Their abundance was not affected by tillage practices or fertility management (organic, conventional). Also, 90 soil samples were analyzed from different Swiss sites categorized as agricultural (wheat and other annual crops) and natural and semi-natural areas (forest and grassland) (Swiss Soil Monitoring Network NABO-project and NRP68-nematode project). Higher numbers of EPN were recorded in natural areas ($P = 0.072$), but this did not translate in higher EPN activity, richness or diversity. In contrast, entomopathogenic fungi were present in higher numbers in agricultural areas ($P = 0.002$), suggesting that they may be a key factor in limiting the native EPN biocontrol potential. Because EPN abundance in cereal plots was very low compared to natural and other agricultural soils, ongoing experiments are exploring if EPN augmentation and alternative management strategies (cover crops) can increase their presence. Optimizing the timing and method of EPN application might provide effective alternatives to repeated conventional applications.

THE GENOME OF THE COLUMBIA ROOT-KNOT NEMATODE (*MELOIDOGYNE CHITWOODI*). **Cha, S.[1], D.A. Humphreys-Pereira[3], A.A. Elling[3], and D.McK. Bird[1,2].** [1]Bioinformatics Research Center and [2]Department of Plant Pathology, NC State University, Raleigh, NC 27695; [3]Department of Plant Pathology, Washington State University, Pullman, WA 99164.

As a genus, root-knot nematodes (RKN: *Meloidogyne* spp.) can likely infect all members of the tracheophyta. However, individual species (and even isolates) have more restricted host ranges; understanding the basis for this restriction will shed light on mechanisms of parasitism, and may inform management decisions. To date, the genomes of three RKN species (*M. hapla*, *M. incognita* and *M. floridensis*) have been published, and data are available for two additional *M. hapla* isolates. To empower a comparative genomics approach, we sequenced and annotated the genome of the Columbia root-knot nematode, *M. chitwoodi*. This nematode is a quarantine species in many countries and is a major pest in potatoes in the Pacific North West of the US. *Meloidogyne chitwoodi* has been reported as sympatric to *M. hapla*, and thus might share machinery for parasitism of specific hosts. Total DNA was extracted from *M. chitwoodi*, race 1 (WAMC1) collected in Washington State. Genomic DNA was sequenced at NCSU on an Illumina MiSeq II instrument. From 20,079,197 paired-end reads, a draft genome was *de novo* assembled into 4,735 contigs (N50 = 82 kbp). We obtained an average genome coverage of 289 reads. Empirical optimization showed the assembly to be extremely sensitive to k-mer size in particular. Accurate gene models for

the highly conserved CEGMA protein set were deduced, and additional models were manually generated from an EST data set. Collectivly, these gene models were used to train Augustus to perform genome-wide gene prediction. CEGMA analysis predicted 245 (98.79%) of the 248 core proteins in the *M. chitwoodi* genome, implying near 100% genome coverage. Conceptual translations of the deduced *M. chitwoodi* transcriptome were compared to the protein sets of *M. hapla*, *M. incognita* and *M. floridensis* using Exonerate software. Consistent with being sympatric, *M. chitwoodi* shared more conserved genes with *M. hapla* than do the tropical species *M. incognita* and *M. floridensis*, even though the latter species have more genes. A thorough characterization and gene classifications based on the Gene Ontology annotations was performed using InterProScan. Currently, analyses are in progress to examine the evolutionary history of the genus *Meloidogyne* and how that relates to, or is derived from, attributes germane to parasitism, such as host range, virulence, fecundity, resistance breaking, survival, and more.

EFFECTS OF OYSTER MUSHROOM COMPOST WASTE ON SOIL AND PLANT HEALTH IN PLANT-PARASITIC NEMATODE INFESTED SOILS. **Ching, S. and K-H. Wang.** 3050 Maile Way, #308, Department of Plant and Environmental Protection Sciences, University of Hawaii Manoa, Honolulu, HI 96822.

Oyster mushroom (*Pleurotus ostreatus*) secretes compounds toxic against nematodes in vitro but has not been examined extensively in vivo. Laboratory and field experiments were conducted to examine the effects of mushroom compost on plant-parasitic nematodes in Hawaii. A Cone-tainer experiment was conducted by amending oyster mushroom compost waste at 50, 33, 2, 1% and 0% (w/w) into 50-cm^3 of yard waste compost to evaluate mushroom compost effect on plant-parasitic nematode penetration into zucchini (*Cucurbita pepo*) roots. The experiment was a completely randomized design with four replications. Each Cone-tainer was inoculated with 200 second stage juveniles (J2) of root-knot nematode (*Meloidogyne incognita*). Mushroom compost amendment did not reduce the number of root penetrations by root-knot nematodes but reduced the viability of the root-knot nematodes in the growing media to 87.5%. The effect of oyster mushroom compost was then evaluated in two basil (*Ocimum basilicum*) field trials for its ability to suppress plant-parasitic nematodes, and to improve soil and plant health. In Trial I, basil seedlings with transplant media amended or not amended with mushroom compost at 50% (w/w) were transplanted into the field plots previously planted or not planted with a cover crop of buckwheat (*Fagopyrum esculentum*) at Poamoho. The basil plants were either drenched or not drenched with mushroom compost water extract every 2 months for 6 months. This 2×2×2 split-split plot experiment was replicated 6 times. Nematodes were monitored every 2 months and plant height and yield were recorded every 2 weeks. Trial II was established at Magoon except that yard waste compost mulch (M) was used to increase soil organic matter instead of planting the buckwheat cover crop. Basil yield was increased by mushroom amendment in Trial I but was only increased in Trial II if applied along with M. Based on repeated measure analysis, mushroom amendment and drenching did not suppress plant-parasitic nematodes in either trials, but increased the abundance of bacterivorous nematodes in Trial I. In Trial II, mushroom amendment improved soil food web structure signify by increased in structure index (SI) regardless of M treatments. With the present of M, mushroom amendment further increased % omnivorous nematodes and nematode richness indicating a better performance of oyster mushroom compost in enhancing soil health especially in soil with higher organic matter. While techniques for field applications of mushroom compost to suppress plant-parasitic nematodes need to be improved, this research confirmed that mushroom compost improved plant and soil health in a basil cropping system.

COLONIZATION OF *GLOBODERA PALLIDA* (PALE CYST NEMATODE) CYSTS BY *TRICHODERMA HARZIANUM*. **Contina, Jn Bertrand[1], G.R. Knudsen[1], and L.M. Dandurand[2].** [1]Soil and Land Resources Division; [2]Plant Science Division, University of Idaho, Moscow, ID 83844.

Globodera pallida, the pale cyst nematode (PCN), has been classified as a quarantine pest in the state of Idaho, USA, where it was first detected in 2006 from a potato processing facility. *G. pallida* can significantly reduce yield of potato in highly infested fields, and may also facilitate secondary infection by plant pathogenic fungi. The fungus *Trichoderma harzianum* shows promise for biological control of plant pathogenic nematodes. *T. harzianum* strain ThzID1-M3 (engineered to express green fluorescent protein, GFP) was tested as a biocontrol agent against different PCN life stages (cysts, eggs and juvenile nematodes). In one experiment, water agar or potato dextrose agar (PDA) plates, each with 25 ppm streptomycin sulfate to inhibit bacterial contaminants, were prepared using standard methods. Plates were inoculated with conidial suspensions of ThzID1-M3 following serial dilution, or with sterile water as a control. Then, ten PCN cysts were placed on the agar surface of each plate. After 10 days incubation (25 °C), colonization of cysts by *T. harzianum* was determined. A second set of experiments was conducted using plastic rhizosphere chambers designed to allow microscopic observation of roots. The dimensions of the chambers were 70 mm x 50 mm x 22 mm, and a corrosion resistant cover glass formed the observation area. Tissue culture potato ('Russet Burbank') germlings were inserted between the cover glass and the filter paper, and the adjacent slot was filled with the soil medium, PCN cysts and homogenized ThzID1-M3 oat inoculum according to the treatments. After 20 days, potato plantlet roots were stained with acid fuchsin dye and observed with epifluorescence microscopy to quantify juvenile nematodes and observe root colonization by ThzID1-M3. The original PCN cysts were

counted, observed microscopically and plated on a *Trichoderma*-selective medium to detect colonization by ThzID1-M3. Results show that *T. harzianum* strain ThzID1-M3 has the potential for biological control of the pale cyst nematode. Increased understanding of the interactions between *T. harzianum*, *G. pallida*, and the host plant, will promote the implementation of a successful biocontrol strategy.

BURKHOLDERIA RINOJENSIS, A NEW BACTERIAL SPECIES WITH BROAD SPECTRUM NEMATICIDE ACTIVITY. **Cordova-Kreylos, A.L., P. Wiese, and A. Stewart.** Marrone Bio Innovations, Inc. 1540 Drew Avenue, Davis, CA 95618.

Burkholderia rinojensis strain A396 is a novel bacterial species, isolated from soil collected under an evergreen tree. A detailed characterization of the microorganism showed that it is not a member of the *Burkholderia cepacia* complex. The microorganism produces a number of compounds with insecticidal activity, and is currently commercialized as Venerate® bioinsecticide. The whole cell fermentation material of the strain has shown nematicidal activity against root-knot nematodes in laboratory bioassays, as well as greenhouse and field trials. Based on initial *in vitro* paralysis testing against *M. incognita* juveniles, we hypothesize that direct effect on nematode J2 (paralysis/mortality) is not the key mode of action of the product. Further testing *in planta* indicated that *B. rinojensis* fermentation broth interferes with gall formation, through a mechanism other than preventing root infection. In field trials with formulated whole cell broth, the product showed acceptable control of burrowing nematode (*Radopholus similis*), spiral nematode (*Helicotylenchus spp.*) and lesion nematode (*Pratylenchus spp.*) in banana; stunt nematode (*Tylenchorhynchus spp.*), lesion nematode (*Pratylenchus spp.*) and lance nematode (*Hopololaimus spp.*) in corn; soybean cyst nematode (*Heterodera glycines*) in soybean, and root knot nematode (*Meloidogyne spp.*) in cucumber. Efficacy was observed both with direct applications as drenches, and when the product was applied as a seed treatment.

RISK ASSESSMENT AND ERADICATION OF *GLOBODERA* SPP. IN U.S. PRODUCTION OF POTATO. **Dandurand, L-M.[1], G. Bryant[2], V. Block[2], W. De Jong[3], D. Denver[4], P. Hutchinson[1], J. Jones[2], J. Kuhl[1], G. Knudsen[1], B. Mimee[5], R. Novy[6], M. Thorton[1], X. Wang[6], J. Whitworth[6], and I. Zasada[6].** [1]University of Idaho, Moscow, ID 83844; [2]James Hutton Institute, Dundee, Scotland; [3]Cornell University, Ithaca, NY 14853; [4]Oregon State University, Corvallis, OR 97331; [5]Agriculture and Agri-Food Canada, Saint-Jean-Sur-Richelieu, Quebec, Canada, J3b 3E6; [6]USDA ARS, various locations.

A transdisciplinary team of researchers including nematologists, plant breeders, extension specialists, and economists were recently awarded a grant by USDA-NIFA Food Security to tackle the ongoing threat of *Globodera* spp. to U.S. potato production. This collaborative effort will be known as GLOBAL (GLOBodera Alliance). Our integrated research, extension, and education efforts will yield a model management approach to protect the U.S. potato industry from current and future introductions of these nematode pests, and will improve U.S. agriculture, food security, and stakeholders' economic interests, knowledge base, and participation in decision-making. The specific objectives of this endeavor will be to 1) Develop and implement effective early warning tools for *Globodera*, including improved detection and diagnosis methods, 2) Use a genomics approach to characterize pathogen virulence and host resistance for development of resistant cultivars, and for detection and identification of effector genes and broader genetic variability in *Globodera* across its geographic range, 3) Identify and deploy potato germplasm conferring resistance to three species of *Globodera* in new economically viable potato varieties, 4) Work with stakeholders and policymakers to co-develop science-based agricultural approaches to deal with the threat of *Globodera* and implement sustainable, environmentally sound agricultural practices for potato production in the context of *Globodera* risk management, and 5) Increase the number of scientists, extension specialists, and educators with the skills and knowledge to effectively address the problem of invasive agricultural pests.

INFLUENCE OF *BRASSICA* COVER CROPS ON ROOT KNOT NEMATODES, SOIL ABIOTIC FACTORS AND PLANT DATA IN POTATO AND TOMATO PRODUCTION UNDER SOUTH AFRICAN CONDITIONS. **Daneel M.[1], H. Fourie[2], E. Engelbrecht[2] and W. Steyn[1].** [1]Agricultural Research Council-Institute for Tropical and Subtropical Crops (ARC-ITSC), Private Bag X11208, Nelspruit 1200, South Africa; [2]North-West University, School of Environmental Sciences and Development, Private Bag X6001, Potchefstroom 2520, South Africa.

Plant-parasitic nematodes cause severe damage to various crops. Brassicaceae cover and/or biofumigation crops have been investigated as an alternative strategy to control these pests. Factors that play an important role in determining efficacy of Brassicaceae control strategies are climate, soil conditions and plant density. A potato and tomato trial were conducted under South African conditions in the North West and Mpumalanga Provinces respectively. *Eruca sativa* (cvs. Rocket Trio (only tomato trial) and Nemat), *Brassica juncea* (cvs. Caliente and Fumigreen (only tomato trial)) and *Raphanus sativus* (cvs. Doublet and Terranova) were evaluated in field experiments for effective *Meloidogyne* control.

Potato (cv. Mondial) and tomato seedlings (cv. Monica) were planted as a follow-up crop in these plots. In the potato trial, results showed that no significant differences were obtained between the four cover crop treatments and the untreated control treatment, however *R. sativus* and *E. sativa* cover crop treatments had lower nematode numbers in roots and tubers than the untreated control treatment while *B. juncea* cover crop treatment had nematode numbers similar to the untreated control treatment. No effect on yield was observed.

In the tomato trial, results showed substantially better nematode control in the *E. sativa* and *R. sativus* treatments compared with UTC and *B. juncea* treatments. This resulted in a significantly higher yield ($P < 0.05$) in both *E. sativa* and *R. sativus*-treated plots compared with untreated control (UTC) and *B. juncea*-treated plots. Correlation analysis confirmed the strong relationship between nematodes and plant data. Statistical analysis further showed that cover crops could explain 70% of plant data variability and 40% of nematode variation. The effect of cover crops and abiotic soil conditions on nematode variability was also investigated and cover crops could explain 31% of the nematode variability while 13% was explained by soil abiotic factors.

Data obtained during this research demonstrated that Brassicaceae crops could be used for their cover- and biofumigation characteristics to reduce root-knot nematode, increase crop yield and contribute towards soil health, however choice of Brassicaceae and follow up crop are important to ensure success.

ATTRACTION OF MELOIDOGYNE SPECIES TO HOST ROOT EXUDATES. **Danquah, W.B., G. Bruening, and V.M. Williamson.** Department of Plant Pathology, University of California, Davis, Davis, CA 95616.

Root-knot nematodes (*Meloidogyne* spp.; RKN) are generally considered the most damaging and economically the most important group of plant-parasitic nematodes worldwide. They can parasitize over 2,000 plant species with a resultant loss in yield due to stunted growth or crop death. Prior to root penetration, RKN infective second stage juveniles (J2) are non-feeding and thus are entirely dependent on their lipid reserves. It is, therefore, essential that they invade a host before these reserves are depleted. However, what attracts nematodes to roots is unknown, even though interference with the root-tip-homing process has the potential to contribute to novel and sustainable protection strategies for crop plants. This study was aimed at discovering the plant-emitted compound(s) that guide the J2 to their entry sites behind the root tip. Using an assay with the thermo-reversible gel Pluronic F-127 (PF127), we showed that J2 of *M. hapla*, *M. incognita* and *M javanica* are highly attracted to root tips and that tomato and *Medicago truncatula* mutants defective in the ethylene-signaling pathway are more attractive than wild type. Cell-free root exudates collected from the root-tip region in PF127 gel caused accumulation of J2 for all three *Meloidogyne* spp., with exudates from the ethylene-insensitive mutants being more attractive than the wild type. This observation suggests that alterations in ethylene signaling modulate levels of attractants and/or repellents. We have developed a novel method of collecting tomato root exudate (TRE) from the terminal 7 mm of seedling root tips. Using an assay based on J2 movement through PF 127 gel, we have demonstrated directed attraction of J2 of *M. javanica* and *M. incognita*, but not *M. hapla*, to this crude TRE. However, following ethyl acetate extraction of acidified TRE, all three RKN spp. were attracted to the aqueous fraction. Size exclusion filtration of the TRE indicates that the attractant is <10,000 kDaltons. Organic fractionation and passage through deionizing columns suggest that the attractant(s) include uncharged, water-soluble plant products. Metabolomic analysis of the attractive fractions and bioassays with individual compounds identified is currently underway to identify the compound(s) responsible for J2 attraction to host roots.

STUDIES ON THE EFFICIENCY AND MODE OF ACTION OF HYPERPARASITE *PASTEURIA* SPP. USED AS A SEED TREATMENT FOR THE CONTROL OF *HETERODERA SCHACHTII*. **Daub M.[1], C. Watrin[2], and R.A. Sikora[3].** [1]Julius Kühn-Institut, Dürener Str. 71, 50189 Elsdorf, Germany; [2]Syngenta Crop Protection LLC, 12085 Research Drive – Suite 185, Alachua FL; [3]Plant Pathology and Protection INRES, University of Bonn, Bonn, Germany 52115.

Chemical or biological nematicides currently are not available for management of the Beet Cyst Nematode (BCN) *Heterodera schachtii* in Europe. Unfavorable conditions for effective cultivation of resistant catch crops drive the excessive use of tolerant sugar beet cultivars with partial resistance in intensive production systems and enhance the risk of virulence selection of local nematode populations. The use of *Pasteuria nishizawae* for the management of BCN was studied in greenhouse experiments with different Pasteuria isolates in cooperation with Syngenta Crop Protection. A susceptible and a tolerant sugar beet cultivar was seed coated with increasing densities of P. nishizawae spores and sown in 400ml pots containing steam sterilized field soil. BCN was added as a 750 J2/100ml suspension 10-14 days after germination. After 250 degree days (>8°C) penetration of BCN in the roots of sugar beets was determined following staining with acid fuchsine. Effects on BCN reproduction were measured by analyzing the final population of eggs and J2 in the cysts of the first generation after 550 degree-days. In comparison to the untreated control P. nishizawae effectively reduced root penetration of BCN in susceptible sugar beet cultivars. Cyst density and reproduction of BCN was significantly reduced in both sugar beet cultivars beginning with low spore densities. In a second ongoing experiment a *P. nishizhawae* isolate was repeatedly inoculated over four BCN-generation cycles aiming to induce suppressiveness in the test substrate in beets grown in 4 Liter containers. Preliminary data show reduced BCN root penetration as well as improved plant performance after the 3rd successive *Pasteuria* inoculation. The results of these studies will be presented.

APPLYING KNOWLEDGE FROM *CAENORHABDITIS ELEGANS* AND BACILLUS SPP. TO NEMATODE INTERACTIONS WITH *PASTEURIA*: IMPLICATIONS FOR BIOCONTROL. **Davies, K.G.** School of Life and Medical Sciences, University of Hertfordshire, College Lane, Hatfield, Hertfordshire, AL10 9AB, UK.

The *Pasteuria* group of endospore forming Gram positive bacteria are parasites of *Cladocerans (Daphnia spp.)* and nematodes and have become an increasingly active area of research. Those Pasteuria spp that infect plant-parasitic nematodes have attracted commercial interest as they are well known to be an important contributor to the development of nematode

suppressive soils and thereby provide an alternative control method to nematicides. The crop protection nematologist wishing to control nematodes has to deal with field populations of nematodes and Pasteuria populations that are genetically mixed and whose genomes will not have been thoroughly sequenced or annotated. One of the key factors in the development of Pasteuria as a biological control agent is its host specificity through what has been hypothesised to be a Velcro-like attachment process; collagen-like molecules on the surface of the endospore are thought to interact with a receptor on the cuticle possibly mediated through mucin-like genes on the cuticle surface coat of the nematode. Recent work shows that not all Pasteuria populations are highly host specific and some populations are more promiscuous than others. This talk will show how closely related and not so closely related genomes both on the nematode side and the bacterial pathogenesis side can throw light of this poorly understood interaction and its implications for the development of a management strategy based on biological control.

TRI-TROPHIC CO-EVOLUTION IN THE *FERGUSOBIA*/*FERGUSONINA*/ MYRTACEAE – A YARN FROM DOWN UNDER. **Davies, K.A.[1], R.M. Giblin-Davis[2], W. Ye[3], S. Scheffer[4], and G.S. Taylor[1].** [1]The University of Adelaide, South Australia; [2]The University of Florida, Davie, Florida; [3]North Carolina Department of Agriculture and Consumer Services, Raleigh, North Carolina; [4]USDA-Agricultural Research Service, Beltsville, Maryland.

The obligate mutualism between *Fergusobia* nematodes and *Fergusonina* flies occurs mainly in Australia, and leads to formation of galls on Myrtaceae. The nematode/fly association is species specific, and each mutualism is generally host plant specific. It is a highly speciose association. The potential of the association as a model for evolutionary studies will be discussed. It appeared to be an exceptional candidate for investigation of cospeciation given the apparent strict vertical transmission of nematodes between fly generations. However, multilocular galls can have multiple conspecific fly foundresses, potentially allowing for horizontal transfer of nematodes. Divergence time estimates for both Myrtaceae and Diptera show that the flies and the plants did not cospeciate at deep levels; and evidence for host-switching will be discussed.

TOLERANCE OF SWEET SORGHUM TO *MELOIDOGYNE INCOGNITA* AND CROP EFFECT ON NEMATODE POPULATION DENSITY. **Davis, R.F., J.E. Knoll, W.F. Anderson, and K.R. Harris-Shultz.** USDA ARS, P.O. Box 748, Tifton, GA 31793.

Sweet sorghum (*Sorghum bicolor*) is a sugar-producing crop that can be used for biofuel and plastics production, and the crop could be incorporated into annual cropping systems in the southern US. The effect of *Meloidogyne incognita* on sweet sorghum yield and sugar content has not been reported. Because sweet sorghum genotypes exhibit a wide range of susceptibility to *M. incognita*, there is the potential for the nematode to affect the yield and sugar content of some genotypes more than others. Field studies were conducted in 2012 and 2013 to determine whether *M. incognita* reduces the yield or sugar content of sweet sorghum. A secondary objective was to evaluate the effect of sweet sorghum on *M. incognita* population levels. Twelve genotypes representing a range of susceptibility to *M. incognita* were grown in a split-plot design where whole-plots were sweet sorghum genotype and sub-plots were either non-treated or fumigated with 1,3-dichloropropene to minimize nematode population levels. Nematode population levels were assessed in early and late season, above-ground biomass (yield) and sugar content (degrees Brix) were measured at harvest, and the percentage yield loss between fumigated and non-fumigated plots of each genotype was calculated. Although there were significant differences among genotypes for both yield and sugar content, there was no effect of fumigation on either variable, nor did fumigation influence the effect of genotype (no genotype × fumigation interaction) on either variable. Because fumigation had no effect on yield for any genotype, the percentage loss did not differ among genotypes. Population levels of *M. incognita* at harvest differed among genotypes and fumigated plots had significantly lower population levels than non-fumigated plots; fumigation influenced the effect of genotype on nematode levels (a significant genotype × fumigation interaction) in 2013 but not in 2012. These results indicate that sweet sorghum is highly tolerant and not significantly damaged by *M. incognita*. However, because some genotypes supported greater population densities of *M. incognita*, which likely increases the risk of damage to subsequent crops, selecting more resistant genotypes for infested fields is recommended.

DEVELOPMENT OF THE SIMULTANEOUS DETECTION OF *MELOIDOGYNE INCOGNITA, M. JAVANICA* AND *M. ARENARIA* BY A MULTIPLEX PCR ASSAY. **Devran, Z.[1], İ. Polat[2], İ. Mıstanoğlu[1], F.G. Göze[3], and M.A. Söğüt[3].** [1]Department of Plant Protection, Faculty of Agriculture, University of Akdeniz, 07058 Antalya, Turkey; [2]Batı Akdeniz Agricultural Research Institute, 07100, Antalya, Turkey; [3]Department of Plant Protection, Faculty of Agriculture, University of Süleyman Demirel, 32260, Isparta, Turkey.

Root-knot nematodes, *Meloidogyne incognita, M. javanica* and *M. arenaria*, cause serious yield losses in protected growing areas in the West Mediterranean region of Turkey.

M. incognita was found the most common root-knot nematode species in the region. Root-knot nematodes could present as mixed population in the protected growing areas. Therefore, we aimed to develop rapid protocol with the capability to simultaneously detect all three species in one run. Initially, multiplex PCR was carried out with three species-specific primers published previously. PCR products were not consistent and appeared as a smear.

Developing of new primers was therefore decided suitable for multiplex PCR. PCR products of inc-K14F/inc-K14R primer set were sequenced in both direction. Forward and reverse primers were designed from the sequences for identification of *M. incognita*. Afterward, these primer sets were screened and checked to *M. incognita* populations. The logical primers were selected for multiplex PCR. Then, the selected primers were used with species-specific primers belonging to *M. incognita* and *M. arenaria* together. The reliability of this multiplex PCR assay was tested using of different biological stages of nematodes in mixed populations related to these species. As a result, we have developed a new multiplex PCR assay to simultaneously detect three species in a single assay. The multiplex PCR could be used to designing of crop rotation modeling in protected growing areas of Turkey and quarantine requirements.

STRUCTURAL APPROACHES TO UNDERSTANDING PLANT AND NEMATODE DEVELOPMENTAL SIGNALS. **DiGennaro, P.[1], B.G. Bobay[2], and D.M. Bird[1,3].** [1]Department of Plant Pathology, NC State University, Raleigh NC 2795; [2]Department of Molecular and Structural Biochemistry, NC State University, Raleigh NC 27695; [3]Bioinformatics Research Center, NC State University, Raleigh NC 27695.

Root-knot nematodes (RKN; *Meloidogyne* spp.) are a pervasive pest of vascular plants and cause substantial crop loss worldwide. Although RKN has been studied for over a century, the molecular basis underpinning the intimate plant-nematode symbiosis remains largely arcane. Emergent technologies, including the generation of tissue specific EST libraries and full genome sequencing, have enabled greater exploration of this phenomenon. However, elucidating specific gene function can often be hindered by a system recalcitrant to traditional genetic manipulation. Based on data mining the RKN genome, we previously showed that RKN uniquely encode multiple families of plant peptide hormones mimics, including CLAVATA-Like Element (CLE) and C-terminal Encoded Peptide (CEP). RKN-encoded peptide mimics are expressed during the parasitic interaction and phenocopy endogenous peptide function *in vitro* including the suppression of lateral roots and the formation of root galls, a hallmark of RKN pathology. To better understand their role in the parasitic interaction, we used structural approaches, including NMR, to establish and compare tertiary structures of plant and nematode encoded peptide hormones. In-solution models reveal family-specific structural properties that have been implicated in receptor binding. This is striking, because the peptide structures were those encoded by RKN and host plants. Molecular dynamics simulations also revealed a high degree of plasticity in the peptide termini which may contribute to unique receptor specificities. To address the latter, we used homology threading algorithms to deduce the tertiary structures of ~300 receptor like-kinase (RLK) ectodomains. This structure-guided modeling approach mapped peptide structures onto a suite of potential receptors, at a resolution sufficient to precisely delineate the electrostatic and hydrophobic points of contact. We observed that the energetics and spatial maps of receptor-ligand docking accurately recapitulate known genetic relationships and resolve conflicting bioassay results. We hypothesize that the unique structural properties among peptides are directly related to their receptor binding characteristics, and discriminate subsequent function. We anticipate refining our models by incorporating additional functional and genetic data as it becomes available. These analyses implicate RKN-encoded plant peptide hormones as core communicative signals in the plant-nematode interaction and constitute a new methodological paradigm in studying effectors at the host-parasite interface.

CAN CONSERVATION OF ENTOMOPATHOGENIC NEMATODES EVER BE PROFITABLE? **Duncan, L.[1], F.E. El-Borai[1], and R. Campos-Herrera[2].** [1]Citrus Research and Education Center, University of Florida, IFAS, Lake Alfred, FL (USA); [2]FARCE Laboratory, University of Neuchâtel, CH-2000 Neuchâtel (Switzerland).

The widespread implementation of practices to conserve predators and parasitoids of aboveground herbivores, contrasts with an absence of tactics to conserve subterranean entomopathogenic nematodes (EPNs). Indeed, more than half a century of EPN research has produced little evidence that endemic communities of these nematodes reliably kill insect pests at especially high rates in some habitats compared to others. Florida citrus orchards are an exception. *Diaprepes abbreviatus* root weevil larvae buried in citrus orchards in the central ridge ecoregion consistently sustain mortality rates higher than 50% per week, due primarily to native EPN species. Orchards in certain flatwoods ecoregions do not support such high levels of natural biocontrol and the regional population levels/economic importance of weevils reflect the regional differences in EPN predation rates. Geospatial surveys of soil properties, EPNs and their natural enemies in both orchards and adjacent natural areas revealed significant relationships between EPN communities and several abiotic variables that affect soil moisture, especially the groundwater depth. The surveys provided little evidence that regional variation of EPN enemies affect EPN spatial patterns. Under controlled conditions in the laboratory, species associated with central ridge and flatwoods ecosystems migrate toward and survive longest in dry and wet soil conditions, respectively, confirming the potential importance of water potential in structuring EPN communities. Field experiments demonstrated that the introduction of sand and any of five EPN species to tree planting holes in an EPN depauperate flatwoods orchard increased root weevil mortality and tree growth and survival. During a period of six years, an undescribed steinernematid nematode that is adapted to, but infrequently encountered in humid flatwoods regions, flourished in the wet, sandy planting hole habitat, whereas species adapted to the central ridge were gradually displaced. Increasing soil porosity is a well-known tactic to increase the insecticidal efficacy of

EPNs. Our results to date suggest that combining this tactic with the introduction of a relatively rare, but effective EPN species may be a method to increase the natural control of weevils in flatwoods orchards to levels approaching that on the central ridge.

VERTICAL DISTRIBUTION OF NEMATODE COMMUNITIES ON THE BARK OF A BLACK WALNUT TREE. **Eisenback, J.D.[1] and V. Paes-Takahashi[1,2].** [1]Dept. of Plant Pathology, Physiology, and Weed Science, Virginia Tech, Blacksburg, VA 24061; [2]Department of Plant Protection, Universidade Estadual Paulista ''Julio Mesquita Filho'' (UNESP/FCAV), Jaboticabal, SP 14884900, Brazil.

A single eastern black walnut tree (*Juglans nigra*) was sampled every 10cm in vertical elevation from ground zero to 212.5cm at the tallest branches. Pieces of bark, 2.5cm in diameter were removed with a battery powered drill fitted with a hole saw. All samples were taken from the northern most side of the trunk, however at very high elevations the branches the entire bark was removed from the branch with a knife, however the surface area was cut to match that of those samples removed with the hole saw. The samples were labeled and placed in open, pint-size plastic storage bags. They were transferred onto Baermann funnels filled with room temperature, tap water; supported with a layer of facial tissue paper; and soaked for 24hr. The nematodes were collected in Syracuse watch glasses and counted according to species. Six major genera were present including *Aphelenchoides* sp., *Cephalobus* sp., *Dorylaimus* sp., *Geraldius* sp., *Laimaphelenchus* sp., and *Plectus* sp. Other genera were also found, however in smaller numbers, and these individuals are currently being identified. *Geraldius* sp. was the most common sp. however *Geraldius* sp. and *Plectus* sp. often occurred together, especially at elevations between 90 and 130cm. Whereas only *Plectus* sp. was found at elevations above 197.5cm. *Laimaphelenchus* sp., *Cephalobus* sp. and *Aphelenchoides* sp. occurred in patches along the major portion of the trunk, and *Dorylaimus* sp. was found only near the base of the tree. All of these species have the ability to become anhydrobiotic and are only active when the bark is wet. Each species probably occupies a unique niche from that of predator, fungivore, or bacterivore. The role that these nematodes play on the tree remains to be determined.

RNA INTERFERENCE AS A NEMATODE CONTROL STRATEGY. **Elling, A.A.** Department of Plant Pathology, Washington State University, Pullman, WA 99164.

RNA interference (RNAi) has developed into an important genetic tool to silence genes in a wide variety of organisms. RNAi technology is firmly established in the model nematode *Caenorhabditis elegans*. An increasing number of studies have aimed at applying RNAi in plant-parasitic nematodes through soaking, but using RNAi as a transgenic trait in plants to control nematodes is still in its infancy. Whereas reducing root-knot nematodes through plant-mediated RNAi has in some cases been very successful, cyst nematode infection could not be equally well managed to date and data for other important nematode genera are largely lacking. The overall RNAi mechanism is well understood but factors to optimize the silencing phenotype remain unknown. Target genes vary greatly in their silencing effect and more efficient targets, ideally with a broader nematode spectrum still need to be identified. This presentation will give a broad overview of the progress in the field to date, discuss strengths and limitations of RNAi technology and focus on silencing the effector gene *16D10* as a specific example to control *Meloidogyne chitwoodi* and other root-knot nematodes. When introduced into stable transgenic potato (*Solanum tuberosum*) lines, silencing of *16D10* gave broad root-knot resistance and reduced the number of egg masses per plant by 65 to 97% ($P < 0.05$) compared to wild type and empty vector controls at 35 days after inoculation with *M. arenaria*, *M. chitwoodi*, *M. hapla*, *M. incognita* or *M. javanica*. Similarly, the number of eggs per plant at 55 days after inoculation was reduced by up to 87% ($P < 0.05$) compared to controls. Plant-mediated silencing of *16D10* was transmitted to *M. chitwoodi* offspring and reduced pathogenicity of nematode offspring on non-RNAi plants. This epigenetic inheritance of the silencing effect could open the door to applications in which transgenic and non-transgenic plants are grown in the same field but are both protected from root-knot nematodes. Overall, host-delivered RNAi is a promising new tool to control nematodes but its efficacy depends on the ability to identify suitable target genes that result in acceptable nematode reduction levels when silenced.

IDENTIFICATION OF NEMATICIDAL PROTEINS IN MICROBES TO DEVELOP NOVEL TRAITS AGAINST PLANT-PARASITIC NEMATODES IN CROPS. **Elling, A.A., R. Box, V. Solis, W. Brandon, K. Schweri, T. Kahn, and J. Daum.** Bayer CropScience, Morrisville, NC 27560.

Plant-parasitic nematodes cause significant damage in a large number of crops worldwide. Our goal is to develop novel nematode resistance traits as part of an integrated nematode control solution that includes transgenic crops, synthetic nematicides and biological control. To identify novel nematicidal proteins, a collection of over 100,000 microbial isolates with diverse ecological and geographic backgrounds was established. Microbial isolates are tested in high throughput bioassays for activity against nematodes, and promising leads are tested on nematodes infecting agronomically important crops. Proteins produced by active microbes are fractionated, isolated, and identified through a series of biochemical steps and multiple rounds of optimization. The genomes of microbial strains that produce nematicidal proteins are sequenced so that the genes that encode active proteins can be cloned. Directed evolution is used to optimize nematicidal effects and

microbial proteins of interest are expressed in agronomically important crops. The resulting plants carrying novel traits are tested for resistance against a broad range of plant-parasitic nematodes under greenhouse and field conditions, and the traits are introgressed into elite germplasm. This screening cascade is very restrictive and only a very small number of microbial strains and genes result in nematicidal traits. The mode of action of active proteins is determined so that traits with multiple modes of action can be combined to enhance the resistance effect. Overall, this approach facilitates identification and characterization of novel resistance traits against diverse plant-parasitic nematodes.

COMPARATIVE GENOMICS/TRANSCRIPTOMICS TO STUDY EFFECTOR GENE BIRTH IN PLANT-PARASITIC NEMATODES. **Eves-van den Akker, S.[1,2], C.J. Lilley[3], E.G.J. Danchin[4], C. Rancurel[4], P.J.A. Cock[5], L.M. Jones[3], H.B. Yusup[3], J.T. Jones[5,6], and P.E. Urwin[3].** [1]Dundee Effector Consortium, University of Dundee, Dundee, UK DD1 4HN; [2]The John Innes Centre, Norwich Research Park, Norwich, UK NR4 7UH; [3]Centre for Plant Sciences, University of Leeds, Leeds, UK LS2 9JT; [4]INRA, Université de Nice-Sophia Antipolis, Centre National de la Recherche Scientifique, Sophia-Antipolis, France F-06903; [5]Dundee Effector Consortium, James Hutton Institute, Invergowrie, Dundee, UK DD2 5DA; [6]School of Biology, University of St Andrews, North Haugh, St Andrews, UK KY16 9TZ.

Plant parasitism in the phylum nematoda has arisen multiple times independently. Within the Tylenchida, it is generally accepted that a linear evolutionary progression occurred from migratory ecto-parasites to migratory endo-parasites, and subsequently from migratory endo-parasites to the highly specialised sedentary endo-parasites. Yet, surprisingly little is known about the genes specifically required for each evolutionary transition. Utilising genome and transcriptome sequencing for free-living and various parasitic nematode species we demonstrate that at least two successive expansions of glutathione synthetase genes occurred during these transition events. The first major expansion increased the reactive oxygen detoxification capacity of the intestine and co-occurred with the evolution of migratory endo-parasitic species. The second major expansion resulted in the evolution of numerous secreted glutathione synthetase effectors, and co-occurred with the evolution of prolonged plant-nematode biotrophic interactions in cyst and reniform nematodes. Despite a diversification in biological function, we demonstrate that glutathione synthetase effectors have retained their biochemical function in glutathione biosynthesis, yet must act in co-operation with abundant plant precursors during the biotrophic interaction. Consistent with this, we detect an accumulation of glutathione within the feeding site during parasitism. Finally, we simultaneously undermine the collective function of all glutathione synthetase effectors by inhibiting the production of their precursors *in planta* at discrete levels by two independent means. This reveals the feeding site-specific glutathione apoptotic threshold and provides an insight into glutathione synthetase effector function. Glutathione synthetase effectors represent a complete description of effector evolutionary origin and a novel mechanism to modulate host redox state and facilitate a sustained biotrophic interaction.

THE USE OF COI MOLECULAR BARCODE COMPARISONS FOR SPECIES DIAGNOSIS OF TWO POPULATIONS OF ROOT KNOT NEMATODES IN INDIANA. **Faghihi, J., D.E. Perla, M. Chen, and V.R. Ferris.** Department of Entomology, Purdue University, West Lafayette, IN 47907.

During a field study on the management of root knot nematode (*Meloidogyne incognita*) in southern Indiana, long known to be a pest of soybeans and other crops, we sequenced the (approximately) 700 base pair area of the mitochondrial gene cytochrome c oxidase subunit one (COI) known as the ''barcode'' area. For some groups of animal taxa, this area of DNA can be used for systematic identification. When we compared our sequence with all Genbank *Meloidogyne* sequences available, it proved to be nearly identical with existing barcode area sequence for *M. incognita*. Subsequently, we recovered root knot nematodes on mint in northern Indiana and determined the COI barcode sequence for these nematodes, which differed from that we had found for *M. incognita*. A comparison with existing Genbank sequences indicated that the specimens from northern Indiana were *M. hapla*. Although the length of the Genbank COI sequences varied greatly among Genbank submissions, a phylogeny that included all available sequences did not differ substantially from other phylogenies based on morphological data or on nuclear molecular data. In our phylogeny, *M. hapla* grouped loosely with *M. graminicola*, and this combined group of taxa grouped most closely with *Meloidogyne incognita*. *Meloidogyne chitwoodi* formed a separate group. In a published phylogeny based on nuclear small subunit ribosomal DNA, *M. graminicula* grouped with *M. chitwoodi*. In our analysis *Bursaphelencus xylophilus* was used as the outgroup species.

USE OF FLUOPYRAM AS A NEMATICIDE IN COTTON. **Faske, T.R., K. Hurd, and M. Emerson.** University of Arkansas, Lonoke Research and Extension Center, Lonoke, AR.

Fluopyram is a SDHI fungicide that is being used as a nematicide in cotton and other row crops. To determine the toxicity of fluopyram to cotton nematodes a few fundamental response assays were conducted. Based on an assay of nematode motility, Meloidogyne incognita and Rotylenchulus reniformis were sensitive to fluopyram. Although nematode paralysis was reversible, low concentrations of fluopyram were effective at reducing tomato root infection by both nematode species. In 2014, two field trials were conducted to evaluate fluopyram as a seed treatment and a liquid

in-furrow treatment for suppression of R. reniformis in cotton. Fluopyram + imidacloprid (Velum Total) applied as an in-furrow treatment was numerically more effective at suppressing nematode reproduction and contributing to a higher yield than fluopyram as a seed treatment alone. Nematode suppression by Velum Total was comparable to Temik; however, the yield benefit was inconsistent between experiments. Given the difference in nematode suppression by the two fluopyram treatments, the movement of fluopyram in soil and onto a developing radicle was investigated. Based on an assay of nematode motility, the majority (P = 0.05) of the fluopyram (30 μg) applied in small soil column remained within 10-cm from the point of application. Similarly, the majority (P = 0.05) of fluopyram on a fluopyram-treated cotton seed (300 μg) remained on the seed coat rather than onto the developing radicle. Though the movement of fluopyram was limited, even in sand, a higher proportion of immotile nematodes were observed from leachate collected 5-10 cm away from the point of application compared to that on the 5-cm long cotton radicle. These data suggest the movement of fluopyram in soil may be an important factor in the suppression of nematode infection along a developing cotton root system. Other studies are underway to better understand use of fluopyram as a nematicide and determine how fluopyram may benefit cotton and other row crop producers.

EXTRACTION OF *ROTYLENCHULUS* SPP. EGGS FROM MAIZE ROOTS USING AN ADAPTED SODIUM-HYPOCHLORIDE METHOD. **Fourie, H., Bekker, S., K.M. Beyers.** North-West University, School of Environmental Sciences and Development, Private Bag X6001, Potchefstroom 2520, South Africa.

Maize is the most important agricultural food crop produced in South Africa. Local nematologists first became aware of the true extent to which *Meloidogyne* spp. were present in production areas during the middle 1990's when an adapted NaOCl method was used to specifically extract eggs and J2 of this genus. However, an increase in what is presumed to be *Rotylenchulus* spp. eggs was extracted together with *Meloidogyne* spp. eggs from maize roots obtained from fields under conservation (CA) and conventional (CT) agricultural practices since the 2012 growing season. Abundance of *Rotylenchulus* spp. females and juveniles in corresponding root and soil samples using other standard extraction methods, complemented suspicions that the visibly smaller eggs extracted with the NaOCl method most probably belonged to this genus. Immature *Rotylenchulus* females were subsequently identified as *Rotylenchulus parvus* by means of morphological identification. From another study conducted during the 2014/15 growing season, the same phenomenon was noted for eggs extracted from roots obtained from a maize field in the Orkney area (North West Province) using the NaOCl method. Subsequently, the width and length of 400 of the two egg types were measured 47 and 70 days after planting. *Meloidogyne* spp. eggs were significantly ($P \leq 0.05$) longer (274±1.07 μm and 274±0.99 μm for the two respective sampling intervals) than those that were presumed to be *Rotylenchulus* eggs, ranging between 155±0.74 (first sampling interval) and 161±0.70 μm (second sampling interval). The latter scenario also applied for the width of *Meloidogyne* spp. eggs that differed significantly ($P \leq 0.05$), ranging between 113±0.48 μm and 114±0.45 μm (first and second sampling intervals, respectively), from those that were presumed to belong to *Rotylenchulus* spp. Width of the latter eggs ranged between 82±0.48 μm and 84± 0.5 μm for the two respective sampling intervals. Since *Meloidogyne* and *Rotylenchulus* spp. have similar feeding habits and life cycles, the adapted NaOCl method used to specifically extract *Meloidogyne* spp. eggs also allows extraction of *Rotylenchulus* spp. eggs. The extent of the distribution of *Rotylenchulus* spp. as well as their population levels thus needs to be assessed in local maize fields to obtain more insight in this scenario. Only then can more accurate conclusions be made about the potential impact of this nematode genus on local maize production and whether it should be regarded as an economically important nematode pest or not.

EFFECTS OF VERMICOMPOST ON RENIFORM NEMATODE (*ROTYLENCHULUS RENIFORMIS*). **Gabour, E.[1], S.P. Marahatta[1], and J-W. Lau[2].** [1]Kauai Community College, 3-1901 Kaumualiĭ Highway, Lihue, HI 96766, [2]University of Hawaii at Manoa, Department of Plant and Environmental Protection Sciences, 3050 Maile Way, Honolulu, HI, 96822.

Vermicompost is known to suppress plant parasitic nematodes such as reniform nematode (*Rotylenchulus reniformis*). However, the effects of vermicompost on reniform nematode is inconsistent. Furthermore, previous results on how vermicompost affects reniform nematode in Hawaiian soils, as well as the most favorable soil application doses, are lacking. Therefore, two laboratory experiments were conducted to determine the required amount of vermicompost for reniform nematode management in Hawaiian pineapple soils, because reniform nematode is especially prevalent in soils where pineapple is farmed. In January 2014 (Trial I) and June 2014 (Trial II), soil samples infested with reniform nematodes were collected from a pineapple field. In Trial I, 1-month-old, and in trial II, 6-month-old vermicomposts were incorporated into the soils at 0%, 0.5%, 1.0%, and 2.0% (w/w) and placed in 10-cm-d plastic pots. Pots were watered and arranged in randomized complete blocks. At the end of 3 weeks, nematodes were extracted from the soil via Baermann funnel and reniform nematodes were counted under an inverted microscope. Soil incorporation of 1-month-old vermicompost at 1.0% reduced reniform nematodes numbers ($P < 0.05$). However, the 6-month-old vermicompost did not reduce reniform nematode numbers ($P > 0.05$). Farmers are recommended to incorporate 1-month-old vermicompost at 1% to their field soils to reduce existing reniform nematodes' populations.

NEXT GENERATION SEQUENCING OF *HETERODERA GLYCINES* FOR TRANSCRIPTOME ASSEMBLY AND POPULATION ANALYSIS. **Gardner, M.[1], E. Davis[2], T. Baum[3], and M. Mitchum[1].** [1]Division of Plant Sciences and Bond Life Sciences Center, University of Missouri, Columbia, MO 65211; [2]Department of Plant Pathology, North Carolina State University, Raleigh, NC, 27695; [3]Department of Plant Pathology and Microbiology, Iowa State University, Ames, IA, 50011.

The soybean cyst nematode (SCN) *Heterodera glycines* is a major pathogen of soybean primarily managed through the use of resistant soybean cultivars. However, repeated use of resistant cultivars selects for populations of SCN able to successfully colonize and reproduce on the roots of these plants. The genes controlling cyst nematode virulence on resistant cultivars have not been identified - a better understanding of nematode virulence on a molecular level can reveal strategies to reduce economic losses from these pathogens. We have developed several inbred populations, including near-isogenic lines of SCN differing in virulence on soybean plants carrying the *Rhg* resistance genes, to aid in the identification of genes involved in gain and loss of virulence. These distinct populations provide a tool with which to investigate the nematode side of the host-pathogen interaction by examining molecular differences that may correlate to their infection phenotype. In order to compare across these populations, a reference transcriptome was generated from early parasitic stages, the first available for *H. glycines*. Sequence assembly resulted in a final transcriptome consisting of 71,093 contigs with a total length of 46.7 Mb. Transcripts encoding stylet-secreted effectors are a point of emphasis in this study, including both known effectors and novel putative effectors identified in the transcriptome. In addition to serving as a mapping reference, the transcriptome also serves as a source of genetic information to mine for further insights into cyst nematode biology.

BIOCLIMATIC MODELING OF SOYBEAN PHENOLOGY AND THE SOYBEAN CYST NEMATODE (*HETERODERA GLYCINES*) LIFE CYCLE IN A CONTEXT OF CLIMATE CHANGE. **Gendron St-Marseille, A-F.[1,2,3], B. Mimee[3], G. Bélair[3], G. Bourgeois[3], and J. Brodeur[12].** [1]Université de Montréal, Montréal, QC, H3T 1J4, Canada; [2]Institut de Recherche en Biologie Végétale, Montréal, QC, H1X 2B2, Canada; [3]Horticulture Research and Development Centre, Agriculture and Agri-Food Canada , Saint-Jean-sur-Richelieu, QC, J3B 3E6, Canada.

Over the past five years, a spectacular 40% growth in soybean total area has occurred in Canada. With this major increase in production, so have nematode pests such as the soybean cyst nematode (SCN). Present in Ontario since 1988, it has been recently detected in the province of Quebec. SCN is the main cause of economic losses in soybean production in Canada and in the United States. Management of SCN is essentially achieved by the use of non-host crops or soybean-resistant cultivars. Because of its ability to survive in soil for an extended period of time, it is virtually impossible to eradicate. For soybean-growing regions located at higher latitudes, a limited number of resistant cultivars are currently available within the maturity groups associated with those photoperiod and temperature regimes. The global warming associated climate change could remodel this host-plant relationship and hamper the sustainability of current management strategies. The anticipation of the effects of climate change on this plant-parasite interaction could promote the development of effective phytosanitary measures to limit its spread. This study proposes the use of bioclimatic models to anticipate the impacts associated with increased temperatures on soybean phenology and on the life cycle of SCN in Quebec by comparing a reference climate period (1971-2000) to three different CO_2 emission scenarios associated with the 2050 horizon (2041-2070). The modelling of SCN life cycle confirms that this species can currently survive in all regions of Quebec, but also that it could complete up to four generations per growing season. Superimposition of past and future soybean distribution maps on the potential reproduction area of SCN shows that it could expand throughout the agricultural area of Quebec. This nematode could colonize all Quebec's ecosystems upon the presence of host plants. The modelling of the SCN life cycle predicts that between four and six generations could be completed, based on latitude and CO_2 emission scenarios. The development and the use of new sources of SCN resistance for the current soybean maturity groups are critical for the management and spreading of this pest in Quebec.

DETERMINING THE ROLE OF PLANT-PARASITIC NEMATODES IN THE ROTATION YIELD EFFECT. **Grabau, Z. and S.Y. Chen.** Southern Research and Outreach Center, University of Minnesota, 35838 120th Street. Waseca, MN 56093.

Corn-soybean crop rotation increases yields of both crops, the phenomena known as the rotation effect. Soybean cyst nematode (SCN, *Heterodera glycines*) is the major pathogen of soybean and a number of plant-parasitic nematodes are associated with corn in the north central United States. The purpose of this study was to determine the role of plant-parasitic nematodes in the rotation effect for corn and soybean and the impact of different crop sequences on these nematodes. Research was conducted at a long-term experimental field site in Waseca, Minnesota. Included in this study were crop sequence treatments in 1 to 5 years of corn monoculture following 5 years of SCN-susceptible soybean, 1 to 5 years of SCN-susceptible soybean following 5 years of corn, continuous monoculture (since 1982) of each crop, and continuous monoculture (since 1982) with non-*Bt* corn cultivar or SCN-resistant soybean since 2010. Granular nematicides (terbufos and aldicarb) have been applied to half of each plot since 2010 to minimize nematode populations across

crop sequences as a way to determine the role of nematodes in the rotation effect. In corn, *Pratylenchus* and *Helicotylenchus* were the major plant-parasitic nematodes at the site. *Pratylenchus* population densities increased significantly ($P \leq 0.05$) in corn monoculture, often incrementally as the number of years in corn increased. Nematicide was effective in reducing *Pratylenchus* populations compared to the treatment without nematicide ($P \leq 0.05$), but no significant role of *Pratylenchus* in the rotation yield effect was detected with the nematicide treatments. *Helicotylenchus* population densities were increased in continuous corn monoculture compared to other corn sequences ($P \leq 0.05$), but *Helicotylenchus* did not appear to have a role in the rotation effect. SCN population densities decreased in corn monoculture and were near or below 200 eggs/100 cm^3 soil, the damage threshold guideline in Minnesota, after 2 full years in corn monoculture. In soybean, nematicide application was not effective enough to prove the role of SCN in the rotation effect. SCN populations did increase while *Pratylenchus* populations decreased significantly ($P \leq 0.05$) in soybean monoculture. *Helicotylenchus* population density decreased in continuous soybean monoculture compared to other sequences ($P \leq 0.05$), but was similar among most soybean sequences. Crop sequences clearly influenced nematode populations, particularly *Pratylenchus* and SCN, but nematicide application was not effective enough to determine if these nematodes influenced the rotation yield effect.

NEMATODES FROM ''THE FIG ISLANDS'' (*FICUS*) AS MODELS FOR STUDYING MORPHOGENESIS AND SPECIATION. **Giblin-Davis, R.M.[1], K.A. Davies[2], N. Kanzaki[1,3], W. Ye[4], and Y. Zeng[5].** [1]Fort Lauderdale Research and Education Center, University of Florida/IFAS, 3205 College Ave, Davie, Florida 33314-7799; [2]The University of Adelaide, South Australia; [3]Forest Pathology Laboratory, Forestry and Forest Products Research Institute, 1 Matsunosato, Tsukuba, Ibaraki 305-8687 Japan; [4]Nematode Assay Section, Agronomic Division, North Carolina Department of Agriculture & Consumer Services, 4300 Reedy Creek Road, Raleigh, NC 27607; [5]Zhongkai University of Agriculture and Engineering, Department of Plant Protection, Guangzhou, 510225, China.

Fig (*Ficus*) species richness is very high, especially near the equator in the lowland tropics. There are over 700 species of *Ficus* in the world with most predicted to have at least one nematode associate inside their mostly aerial figs (sycones). Overall nematode diversity inside sycones follows the latitudinal gradient by default, i.e., there are generally similar numbers of nematode species per fig, but more fig species near the equator leading to increases in overall fig nematode diversity closer to the equator. Because nematodes are aquatic animals, most species that have developed associations with the aerial sycones of figs have run a selective gauntlet to become linked with fig host biology and their mutualist pollinators and transport hosts (the figwasps). Once successfully associated with figs and figwasps, nematode lineages experience barriers to gene flow that could lead to valuable examples of geographical speciation (association by descent), sexual or ecological divergence, competitive speciation, or cospeciation. Host range mistakes by a transport host and extinction through accidents or competition can potentially cloud the phylogeographic story. Yet, the abiotic isolation and potential for internal sycone niche similarity/uniformity for associated nematodes makes the system potentially less complicated for study than typical soil or marine nematode ecosystems in terms of gene flow and some other factors. We discuss general observations from more than a decade of global survey and systematics research concerning an increasing array of nematode lineages relative to morphogenesis and speciation. The main lineages that have become associated with ''the fig islands'' appear to involve the Tylenchomorpha (three fig-specific and one generalist lineages of Aphelenchoididae, with one apparently fig-specific ''anguinid'' lineage), the Diplogastromorpha (several fig specific lineages, and several generalist genera), and the Rhabditomorpha (one generalist genus). Although most fig-nematode associates are not culturable, the main niches that they fill inside the sycone appear to involve (or have involved) gradients from mycophagy to obligate plant-parasitism, and saprophagy to insect parasitism or predation.

NEW NON-FUMIGANT CONTACT NEMATICIDE, NIMITZ, REGISTERED BY EPA. **Navia Gine, P.A. and H.S. Young.** ADAMA, 3120 Highwoods Blvd. 100 Raleigh, NC 27604.

On September 14, 2014, NIMITZ™ received EPA registration for nematode control on fruiting vegetables and cucurbits. NIMITZ, an efficacious non-fumigant nematicide, provides control of plant-parasitic nematodes with simple application features and unmatched user safety. With its 'CAUTION' signal word, using NIMITZ only requires gloves and long-sleeve PPE. The product's active ingredient, fluensulfone, has a unique mode of action which categorizes it within a new chemical class. The United States is the first country to receive registration. NIMITZ requires no Fumigant Management Plans, no 24-hour field monitoring, no buffer zones, no re-entry interval (REI), a 7-day pre-plant interval, and minimal Personal Protective Equipment (PPE). NIMITZ causes irreversible nematicidal activity resulting in pest mortality within 24 to 48 hours. NIMITZ provides nematode control competitive with the best commercial nematicides, but has a safer toxicological profile. The residual activity of NIMITZ is visibly seen in gall-free and decay-free root systems, often lasting season-long. Application options for NIMITZ include drip-injection, and broadcast or banded soil-sprays with mechanical incorporation. The first EPA registration includes: cucumbers, watermelons, cantaloupe, squash, tomatoes, peppers, okra and eggplants. NIMITZ is currently registered in the 26 primary vegetable producing states and Puerto Rico. Multiple country registrations are pending.

EFFICACY OF CHLOROTHALONIL AGAINST PLANT PARASITIC NEMATODES ON GOLF COURSE BERMUDAGRASS. **Gu, M., and W.T. Crow.** University of Florida, Gainesville, FL 32611, USA.

Belonolaimus longicaudatus (Sting nematode) and *Meloidogyne graminis* (Root-knot nematode) are considered major destructive plant parasitic nematodes on golf course bermudagrass in Florida. Nematicide application is the most widely adopted method for controlling plant parasitic nematodes on golf course greens. However, the loss of many nematicides used in previous decades has brought forward the need for new nematode management tools for use on turfgrass. This study evaluated the efficacy of a fungicide, chlorothalonil, against *B. longicaudatus* and *M. graminis* in greenhouse experiments. Additionally, field trials evaluated chlorothalonil for ability to improve efficacy of abamectin against *B. longicaudatus* and *M. graminis* on golf greens. In the greenhouse, chlorothalonil had efficacy against *B. longicaudatus*, but not *M. graminis*. In field trials, applying chlorothalonil in conjunction with abamectin increased efficacy against *B. longicaudatus* but not *M. graminis,* compared to abamectin without chlorothalonil. Further, chlorothalonil enhanced turf percent green cover and root length when applied in conjunction with abamectin compared to abamectin alone. These results indicate that chlorothalonil has efficacy against certain plant-parasitic nematodes, such as *B. longicaudatus* and helps improve the turf benefits of abamectin. Currently the mode of action of chlorothalonil on *B. longicaudatus* is unknown.

INTEGRATING THE CONCEPTS OF FERTILIZER USE EFFICIENCY (FUE) AND NEMATODE-BASED SOIL FOOD WEB MODELS FOR BROADER USE IN SOIL HEALTH MANAGEMENT. **Habteweld, A.W.[1,2], D. Brainard[2], M. Ngouajio[5], S. Kravchenko[3], P.S. Grewal[4], and H. Melakeberhan[1,2].** [1]Agricultural Nematology Laboratory; [2]Department of Horticulture, Michigan State University, East Lansing, MI 48824, USA; [3]Department of Plant, Soil and Microbial Sciences, Michigan State University, East Lansing, MI 48824; USA, [4]Department of Entomology and Plant Pathology, University of Tennessee, Knoxville, TN 37996, USA; [5]Division of Plant Systems-Production, USDA-NIFA, Washington, DC 20024, USA.

The Fertilizer Use Efficiency (FUE) model separates the relationships among plant growth, herbivore nematode parasitism, nutrient deficiency and toxicity (Melakeberhan, 2006), and allows cross disciplinary and integrated decision-making when amending soil. It calculates treatment outcome as a percent of control (untreated check) and graphically expresses data in four quadrants from best (A) to worst case (D) scenarios. The FUE model was modified to include assessment of beneficial nematodes and soil physicochemical properties in four quadrants (E, F-best case, G, worst case, and H, (Melakeberhan and Anvendaño, 2008). In order to fully assess soil health, the modification needs the power of integrating nematode functions. The objective of this study was to integrate weighted nematode guilds into the FUE model and test if the outcomes overlap with Quadrant F, which corresponds with Quadrant B of the Ferris *et al.* (2001) soil food web model. We tested the effects of plant and animal waste compost applied at 135, 203 and 270 kg N/ha to a processing carrot (*Daucus carota*) cv 'Cupar' field in 2012-2014. Urea and non-amended check were included as controls. Nematodes were extracted from soil samples collected at planting and harvest, enumerated and assigned to c-p 1 to c-p 5 groups. We adopted the Ferris *et al.* (2001) c-p value weighting for c-p 2 to c-p 5 nematodes and added weight for c-p 1 nematodes using the same formula, $0.8*(0.5*(n+1))^2$ where n is the c-p value and '0.5' is a fraction of increase in food web complexity with each increment in c-p class. This gives c-p value weighting of 0.2, 0.8, 1.8, 3.2 and 5 for c-p 1 to c-p 5, respectively. Weighted nematode guild abundance was calculated by multiplying the number of c-p group abundances with their respective c-p group weight and combined to form one value. The results were then fitted to the FUE model relative to soil organic matter (OM) and marketable carrot yield. Data points for OM and marketable carrot yield from animal and plant compost amendments appeared in quadrant F. This shows not only an improvement of the FUE model for soil health analysis, but integrates it with the soil food web model. The implications of this modification for much broader and cross-disciplinary analyses of soil conditions will be discussed.

IMPACT OF COMPOST AMENDMENTS ON SOIL FOOD WEB, SOIL PHYSICOCHEMICAL PROPERTIES AND CARROT YIELD. **Habteweld, A.W.[1,2], D. Brainard[2], M. Ngouajio[5], S. Kravchenko[3], P.S. Grewal[4], and H. Melakeberhan[1,2].** [1]Agricultural Nematology Laboratory; [2]Department of Horticulture, Michigan State University, East Lansing, MI 48824, USA; [3]Department of Plant, Soil and Microbial Sciences, Michigan State University, East Lansing, MI 48824, USA; [4]Department of Entomology and Plant Pathology, University of Tennessee, Knoxville, TN 37996, USA; [5]Division of Plant Systems-Production, USDA-NIFA, Washington, DC 20024, USA.

This study tested the hypotheses that compost amendment would enhance soil food web structure index (SI), improve soil biological and physicochemical properties, and increase carrot (*Daucus carota*) yield and quality, but these effects would differ by compost type and rate of application. Plant (PC) and animal waste (AC) compost was applied to supply 135, 203, and 270 kg N/ha for a processing carrot cv 'Cupar'. Urea (UR) and non-amended check (CK) were included as controls. Nematode community was analyzed from soil samples collected at approximately 4- week intervals in 2012-2014 growing seasons. Soil respiration (SR) and physicochemical properties were determined from soils collected at planting and harvest. Compost amendments except AC at 203 kg N/ha significantly ($P < 0.05$) increased SI at 132 days after planting (DAP) in 2012 compared with the rest of 2012 sampling dates in Cupar, but this effect was not observed in UR and CK. At 133 DAP in 2013, all treatments significantly increased ($P < 0.05$) SI compared with the previous sampling dates in 2012 and 2013. Compost

amendments significantly increased P and pH compared with UR while UR significantly increased NO_3-N compared with compost amendments at the end of 2013 growing season. There was no difference in total yield and carrot quality among treatments in Cupar. Multiple factor analysis (MFA) showed positive correlation between SI and calcium, magnesium, cation exchange capacity and soil pH while negative correlation was observed for total unmarketable carrot yield in which dimension 1(33.30 %) and dimension 2 (12.60 %) represent the first and second best summary of the variability of the information. Overall, results suggest that compost amendments improved SI compared with UR. Compost amendments also increase pH in average from 7.2 to 7.7 and phosphorus in average from 53 to 61 ppm after two years. MFA result supports the importance of SI as an indicator of soil health and carrot quality. Overall, results support the tested hypotheses and suggest that the compost-based improvements in soil food web structure may lead to increases in ecosystem services provided by the soil food web.

NEW CHEMISTRIES AND COMPOUNDS FOR NEMATODE MANAGEMENT IN POTATOES. **Hafez, S.L. and C. Sevy.** University of Idaho Parma Research and Extension Center, 29603 University of Idaho Lane, Parma, Idaho 83660.

New chemistries and compounds were tested and evaluated for efficacy as nematicides under field conditions at the University of Idaho Parma Research and Extension Center in Parma, Idaho, USA. Tested materials included: Movento, Nimitz (MCW-2), Varnimo, SP#25, SP#26, BCS-CS#, BCS-AR#, Q8#, BioAct, VBC# and Metam CLR. Chemicals were tested for efficacy either as a standalone product, or in combination with a Vydate standard or multiple chemicals. Efficacy was tested on root-knot (CRKN) or root-lesion (RLN) nematode. Applications were made by hand broadcast, 4-6'' band in-furrow at planting, chemigated or applied via foliar applicator. Evaluations consisted of total yield, marketable yield and percent infection for CRKN and total and marketable yield for RLN. Statistical comparisons were made between treatments and untreated control.

Standalone treatments consisted of Nimitz at varying rates, SP#26, BCS-AR#, VBC# at varying rates and application timings, Varnimo, BioAct and Metam. Nimitz treatments increased overall yield by 5-26% and decreased infection by 14-19%. SP#26 increased overall yield 6% and decreased infection by 4%. BCS-AR# increased overall yield by 15% and decreased infection by 8%. VBC at varying rates and application times showed a -11% to 14% change in overall yield and 0-21% infection decrease. Varnimo decreased overall yield by 19% but still lowered the infection by 20%. BioAct increased overall yield by 15% and decreased infection by 6%. Metam increased overall yield by 33% and decreased infection by 69.2%. Most trials resulted in a decrease in infection (0-69.2%).

Vydate program treatments usually consisted of an application of test chemical at planting and several Vydate chemigations throughout the season. Tested chemicals consisted of Nimitz, BCS-CS#, BCS-AR#, BioAct and Q8#. Nimitz treatments increased overall yield 3-10% and decreased infection by 15-19%. BCS-CS# changed overall yield 20-27% for liquid formulation and -10-4% for granular formulation. Both liquid and granular formulations decreased infection 17-31%. BCS-AR# increased overall yield by 6% and decreased infection by 28%. BioAct increased overall yield by 6% and decreased infection by 20%. Q8# reduced overall yield -35-2% but still decreased infection by 8-18%. All treatments resulted in a decrease in percent infection (8-31%).

Other management programs consisted of a multiple chemical combination, usually another numbered compound or chemistry, Adsorb or Movento. Tested chemicals consisted of BCS-AR#, Varnimo, SP#25, Metam CLR, Movento and BioAct. The BCS-AR# program increased overall yield by 4% and decreased infection by 27%. The Varnimo program decreased overall yield by 7% and decreased infection by 17%. The SP#25 program increased overall yield by 4% and decreased infection by 22%. The Metam CLR + Adsorb program increased overall yield by 14-26% and decreased infection by 72.6-74.3%. The Movento program increased overall yield by <1% and decreased infection by 21%. The BioAct program increased yield by 30% and decreased infection by 27%. All programs decreased infection (17-74.3%) Most chemicals in all treatments decreased infection (0-74.3%) and most treatments increased overall yield (-35 to 33%).

EFFECT OF MOVENTO ALONE OR IN COMBINATIONS WITH OTHER NEMATICIDES ON *MELOIDOGYNE CHITWOODI* AND *HETERODERA SCHACHTII* ON SUGAR BEET. **Hafez, S.L.[1], C. Sevy[1], K. Luff[2], and P. Sundararaj[3].** [1]U of I Parma REC, 29603 U of I Lane, Parma, ID 83660, USA; [2]3554 East 4000 North, Kimberly, ID, 83341, Bayer CropScience; [3]Bharatiar University, Coimbatore-641046, Tamilnadu, India.

Experiments were conducted to study the efficacy of Movento alone or in combinations with other nematicides on *Meloidogyne chitwoodi* on potato and *Heterodera schachtii* on sugar beet at the University of Idaho, Parma Research and Extension Center, Parma, Idaho. The experiments were carried out in a randomized complete block design with thirteen treatments for potato and six treatments for sugar beet. For potato experiment, Vapam was applied by a commercial applicator and Vydate @ 4.2 pt/ac was applied at planting by using a CO_2 power plot sprayer. Foliar application of Vydate@ 2.1 pt/ac was repeated starting on 28 Jun 2011 and every 14 days intervals afterward for a total of up to5 applications depending on treatments using plot chemigation sprinklers. Movento @ 3.33 or 5 oz/ac was applied starting at sufficient foliage at 2 weeks interval for a total of up to 5 applications depending on treatments. Foliar applications of Movento were made by using handheld CO_2 power plot sprayer. For sugar beet experiment, Temik 15G @ 20 + 13 lb/A was applied at planting and side dressed. Movento @ 5 fl oz/A plus MSO @ 0.25 % v/v as a spray mix was applied at different intervals.

Movento was applied by using a handheld CO_2 powered plot sprayer. Potatoes and sugar beet were harvested at maturity and the yield data were recorded. The clean tuber yield was significantly higher in all treatments compared to untreated control. The infected tuber yield was significantly lower in all treatments as compared to untreated control except Movento applied at 56 and 70 days after planting. The significantly lowest infected tuber was obtained in treatment combination of Vapam 37.5 gal/ac applied on 16 Nov 2010, and Movento 5 oz/ac at 56 and 70 day after planting. Movento applied three times at 21, 35 and 49 DAE significantly increased beet yield as compared to Movento applied four times at 21, 35, 49 and 63 DAE but not different from other treatments.

EFFECT OF DIFFERENT RATES OF NIMITZ ALONE OR IN COMBINATION WITH OTHER NEMATICIDES ON *MELOIDOGYNE CHITWOODI* ON POTATO AND *HETERODERA SCHACHTII* ON SUGARBEET. **Hafez, S.L.[1], P. Sundararaj[2], and C. Sevy[1].** [1]U of I Parma REC, 29603 U of I Lane, Parma, ID 83660, USA; [2]Bharatiar University, Coimbatore-641046, Tamilnadu, India.

Two experiments were carried out to determine the effect of different rates of Nimitz alone or in combination with other chemicals on Columbia root-knot nematode on potato and sugar beet cyst nematode on sugar beet at Parma Research and Extension Center, Parma, Idaho. Both the experiments were laid out in a completely randomized design with eight treatments including untreated control each with five replications in a silt loam field. For the first experiment, Nimitz 15G @ 12, 18, or 24 lbs/A was broadcasted, incorporated over the bed 1 week before planting. Potato cv Ranger Russet seed pieces were planted on 8 May 2014. Vydate C-LV @ 4.2 pt/A was applied in furrow at planting and chemigated @ 2.1 pt/A starting at 800 DD on 6/18 and biweekly for a total of 6 applications. For the second experiment, sugar beet seeds were sown in the field on 29 Apr 2014. Temik 15G @ 20 + 13 lb/A was applied at planting and side dressed on 26 May. Liquid formulation-Nimitz 480 EC @ 3.6, 5.4 or 7.2 pt/A or granular formulation-Nimitz 15G @ 12, 18 or 24 lb/A was broadcasted, incorporated one week before planting. Potato tuber yield was significantly higher in Nimitz applied alone @ 18 and 24 lb/A as compared to untreated control. Infected tuber yield were significantly decreased in all treatments (17-11%) as compared to untreated control (31%). Liquid formulation of Nimitz @ 7.2 pt/A and granular formulation @ 18 lb/A significantly decreases beet yield as compared to untreated control indicating that the product might have phytotoxicity on sugar beet. The effect of Nimitz may be either masked by the high pressure of nematode in the field or by its phytotoxicity effect.

HOST PREFERENCE AND SEED-BORNE TRANSMISSION OF THE STEM NEMATODES, *DITYLENCHUS WEISCHERI* AND *D. DIPSACI* UNDER GREENHOUSE CONDITIONS. **Hajihassani, A.[1], M. Tenuta[1], and R.H. Gulden[2].** [1]Department of Soil Science; [2]Department of Plant Science, University of Manitoba, Winnipeg, MB, Canada R3T 2N2.

The stem nematodes, *Ditylenchus* spp., are migratory endoparasitic nematodes with potential to reduce production of many agricultural crops. The stem and bulb nematode, *D. dipsaci*, is a quarantine parasitic species of many cultivated crops especially in temperate climates. Recently, we reported the presence of another stem nematode, *D. weischeri*, on creeping thistle (*Cirsium arvense*) in the Canadian Prairies. Little is known concerning the pathogenicity and host-suitability of *D. weischeri*. Therefore, two greenhouse studies were carried out to a) evaluate the host preference of *D. weischeri*, isolated from creeping thistle in the Canadian Prairies, and *D. dipsaci*, isolated from garlic in Ontario (study 1), and b) determine if these nematodes are capable of being seed-borne transmissible using yellow pea and creeping thistle (study 2). For study 1, creeping thistle, garlic, five yellow pea, three common bean, two chickpea varieties and one variety of lentil, spring wheat and canola were examined. Plants were inoculated with either 100 (study 1) and 1000 (study 2) juveniles of either *D. weischeri* or *D. dipsaci* in 15µl of 1.5% carboxymethyl cellulose (CMC). Before extraction for the nematodes, plants were grown for eight weeks or until they were matured in study 1 and 2, respectively. Plants inoculated with CMC alone were as controls. The results of study 1 *showed that* only two (Agassiz and Golden) out of five yellow pea varieties were weak hosts of *D. weischeri* with a reproduction factor (R_f) of slightly greater than 1. Creeping thistle was an excellent host for *D. weischeri* having an R_f of 5.4. All other crops examined were not hosts ($R_f < 1$) for *D. weischeri*. All five yellow pea varieties (range $R_f = 2.1$ to 6.9), three common bean (1.4 to 2.4), one chickpea variety (Frontier, 1.3) as well as garlic (6.2) were hosts for *D. dipsaci* but lentil, spring wheat, canola and creeping thistle were not. The results of study 2 *demonstrated that D. weischeri was not a seed-borne parasite of yellow pea, unlike, D. dipsaci which was recovered from throughout plant tissues toward the seed-pod and with seed. Conversely D. weischeri and not D. dipsaci was recovered from creeping thistle seeds*. Pulse and non-pulse crops are not preferred host for *D. weischeri*, unlike *D. dipsaci* which is potential parasite of pulse crops tested.

OLFACTORY BEHAVIORS OF PARASITIC NEMATODES. **Hallem, Elissa A., J. Lee, M.L. Castelletto, and S.S. Gang.** Department of Microbiology, Immunology, and Molecular Genetics, University of California, Los Angeles, Los Angeles, CA 90095.

Many parasitic nematodes actively locate hosts to infect, yet their host-seeking behaviors are poorly understood. We are studying how parasitic nematode infective juveniles (IJs) use olfactory cues to identify hosts, and how host-seeking behaviors differ in species with different lifestyles and host ranges. We are investigating these questions in both entomopathogenic nematodes (EPNs) in the genera *Heterorhabditis* and *Steinernema*, and skin-penetrating mammalian-parasitic nematodes such as the human parasite *Strongyloides stercoralis*.

In the case of EPNs, we find that they are attracted to carbon dioxide (CO_2) as well as a diverse array of insect and plant odorants. Moreover, CO_2 is an essential host cue for many EPNs: attraction to hosts is greatly reduced in the absence of CO_2. In addition, the olfactory preferences of many EPNs depend on both IJ age and cultivation temperature. For example, many odorants are attractive to IJs cultivated at lower temperatures but repulsive to IJs cultivated at higher temperatures, and vice versa. Temperature-dependent changes occur in individual IJs and are reversible, indicative of olfactory plasticity. The extent to which EPNs display olfactory plasticity varies across species. For example, *Steinernema carpocapsae* displays both temperature- and age-dependent changes in olfactory preferences, while some species appear to show primarily age-dependent changes and still others show little or no olfactory plasticity. Many EPNs are found in geographical regions that undergo seasonal temperature variation, and olfactory plasticity may enable them to optimize host seeking under changing environmental conditions. A better understanding of host seeking by EPNs may lead to new strategies for enhancing their efficacy as biocontrol agents.

In the case of skin-penetrating nematodes, we find that they are not attracted to CO_2 but are attracted to a diverse array of skin and sweat odorants. Many of the strongest attractants for *S. stercoralis* are also known mosquito attractants, suggesting that human-parasitic nematodes and mosquitoes target humans using many of the same olfactory cues. Olfactory preferences of *S. stercoralis* differ between the IJ stage and the free-living life stages, suggesting a mechanism by which host seeking is specific to IJs. A comparison of odor-driven behavior across mammalian-parasitic, insect-parasitic, and free-living species demonstrated that parasite olfactory preferences reflect host specificity and infection mode rather than phylogeny, suggesting an important role for olfaction in the host-seeking process. We are now elucidating the neural circuits and signaling pathways that mediate host seeking in *S. stercoralis* using calcium imaging, genome editing, and quantitative behavioral analysis. A better understanding of host seeking by human-parasitic nematodes may lead to new strategies for preventing harmful nematode infections.

VARIATION OF NEURONS IN THE VENTRAL NERVE CORD AND DII-FILLING PATTERNS IN NEMATODE SPECIES. **Han, Z. and N.E. Schroeder.** Department of Crop Sciences, University of Illinois, Urbana, IL 61801.

All nematodes have a nervous system to regulate behavior. Sophistic behavior is required for nematodes to complete the life cycle. Although the habitats of nematodes are diverse, the nerve system is considered conserved. Much of our understanding of nematode neurobiology is influenced by studies in two nematodes: the free-living species *Caenorhabditis elegans* and the mammalian gastrointestinal parasite *Ascaris suum*. The two distinct species *C. elegans* and *A. suum* share many similarities in neuroanatomy. We examined species across several clades and found variation in the numbers of neurons in the ventral nerve cord (VNC), and staining patterns of certain sensory neurons. With nuclear staining using DAPI, we found *Sternenema carpocapsae* and *S. glaseri* had 40% more neurons in the VNC than *C. elegans*, whereas *Aphelenchus avenae* and *Heterodera glycines* had 20% more neurons in the VNC than *C. elegans*. In *C. elegans*, six pairs of amphid neurons, two pairs of phasmid neurons and six inner labial neurons can be stained using fluorescent dyes. Using a common staining method DiI-filling, we found different staining patterns in several species compared to *C .elegans*. In *Pratylenchus penetrans*, only one pair of amphid neurons was DiI-filled and no phasmid neurons were DiI-filled. In *S. carpocapsae* and *S. glaseri*, six inner-labial-like neurons were DiI-filled, but DiI-filled amphid neurons were rarely observed. Various numbers of neurons in the VNC and different staining pattern of DiI-filling in the examined species suggest that variation in nematode neuroanatomy exists.

REDESCRIPTION AND MOLECULAR CHARACTERIZATION OF XIPHINEMA CHAMBERSI THORNE, 1939 (NEMATODA: LONGIDORIDAE) FROM LIVE OAK TREES IN JEKYLL ISLAND, GEORGIA WITH COMMENTS ON ITS MORPHOMETRIC VARIATIONS. **Handoo, Z.A.[1], L.K. Carta[1], A.M. Skantar[1], S.A. Subbotin[2], and S.W. Fraedrich[3].** [1]Nematology Laboratory, USDA, ARS, Beltsville, MD 20705; [2]California Department of Food and Agriculture, Plant Pest Diagnostic Center, 3294 Meadowview Road, Sacramento, CA 95832; [3]Forest Service, Southern Research Station, 320 Green Street, Athens, GA 30602.

A population of *Xiphinema chambersi* from the root zone around live oak (*Quercus virginiana* Mill.) trees on Jekyll Island, Georgia, USA is described using both morphological and molecular tools and compared with descriptions of type specimens. Initially, because of few morphological differences this nematode was thought to represent an undescribed species. However, upon further examination, the morphometrics of the nematodes from live oak tend to agree with most of the morphometrics in the original description and redescription of *X. chambersi* except for differences in V% relative to body length, slightly shorter stylet length, different c value and the number of caudal pores. We consider these differences to be part of the normal variation within this species and accordingly re-describe and image this new population of *Xiphinema chambersi* from the roots of live oak. It is characterized by having females with a body length of 2.1-2.5 mm; lip region slightly rounded and set off from head; total stylet length 170-193μm; vulva at 20.4%-21.8% of body length; a monodelphic, posterior reproductive system; elongate, conoid tail with a blunt terminus and 4 pairs of caudal pores, of which 2 pairs are subdorsal and 2 subventral. Sequence data from the D2-D3 region of the 28S rDNA molecule subjected to GenBank sequence comparison using BLAST showed that the sequence had 96 and 99% similarity with *X. chambersi* from Alabama and Florida,

respectively. Phylogenetic relationships of *Xiphinema chambersi* with other xiphinematids based on analysis of this DNA fragment are presented. This finding represents a new location of *X. chambersi* in Gorgia and a new host record of live oak for this species. Additional information regarding the distribution of this species within the region is anticipated.

EFFECTS OF SUNN HEMP FOLIAGE AND MACADAMIA NUT HUSKS ON PLANT-PARASITIC AND BENEFICIAL NEMATODES. **Henmi, V.H. and S.P. Marahatta.** Kauai Community College, 3-1901 Kaumuali´i Highway, Lihue, HI 96766.

Sunn hemp, *Crotalaria junce*a, foliage (SH) has been documented as a soil amending material for controlling multiple plant-parasitic nematodes such as root-knot nematode, *Meloidogyne* spp., burrowing nematode, *Radopholus similis*, or reniform nematode, *Rotylenchulus reniformis*, and enhancing beneficial free-living nematodes. However, several Hawaiian farms are using macadamia nut, *Macadamia integrifolia*, husk (MN) as a mulch and soil amending material in orchards. Thus, a comparative evaluation of SH and MN was conducted to determine the effects of SH and MN on *Meloidogyne*, *R. similis*, and beneficial nematodes through two laboratory experiments. In both experiments, banana orchard soils infested with *R. similis* and *Meloidogyne* were sampled. Soils were placed into 12 7.62-cm-d planter pots. Each pot was filled with 300 cm^3 soil and immediately amended with 1% (w/w) SH, MN or not amended (CC). Pots were arranged in randomized complete blocks with four replications. At the end of each experiment, nematodes were extracted through the Baermann funnel technique. In both trials, SH consistently showed the highest number of beneficial nematodes ($P < 0.05$), and the lowest population trend of *R. similis*. The effects of SH on *Meloidogyne* were not observed in Trial-I. However, in Trial-II, *Meloidogyne* were not found in SH. Compared to CC and SH, MN did not increase beneficial nematode number ($P > 0.05$), nor reduce *R. similis* and *Meloidogyne* populations ($P > 0.05$). Hawaiian farms should not choose MN as an alternate to SH for *Meloidogyne* and *R. similis* suppression and beneficial nematode enhancement.

ANALYSIS OF SOILS AND PLANT PARASITIC NEMATODES AT STRAWBERRY AND WATERMELON CULTIVATION IN KOREA. **Heon-Il, K.[1,2], Y.J. Ko[1,2], G. Eun[1,2], N.S. Park[2], D.G. Kim[3] and I.S. Choi[1,2*].** [1]Dept. of Plant Bioscience, Pusan National University, Miryang, 627-706, Korea; [2]Nematode Research Center, Life and Industry Convergence Research Institute, Pusan National University, Miryang, 627-706, Korea; [3]Organic Agriculture Research Institute, Gyeongsangbukdo Agricultural Research & Extension Services, Uiseong, 769-803, Korea.

A survey was conducted to determine the occurrence and population density of plant-parasitic nematodes in strawberry and watermelon cultivation field. Soil samples, we used for isolation nematodes, were collected at major strawberry and watermelon plantation in Yeongnam province. And analyzed the correlation between soil pH, electrical conductivity (EC), organic matter and soil texture. Among the 112 soil samples in strawberry cultivation field, 46 parts were of the *Meloidogyne* spp., which was most commonly found at 41%, and 36 parts were *Pratylenchus* spp., which comprised 32% of the samples, and 20 parts were of *Helicotylenchus* spp., which made up 18% of the samples. Among the 36 soil samples in watermelon cultivation field, 19 parts were of the *Meloidogyne* spp., which was most commonly found at 53%, and 13 parts were *Pratylenchus* spp., which comprised 36% of the samples, and 8 parts were of *Helicotylenchus* spp., which made up 22% of the samples. The root-knot nematode(*Meloidogyne* spp.) was most commonly found at soil samples of strawberry and watermelon. It was found that there was no correlation between soil pH and the density of root-knot nematodes. Besides, it was also found that there was no correlation between EC and the density of root-knot nematodes. The result of the correlation between density of root-knot nematode and organic matter turned out as $y=1392.7$ $R^2=0.6647$ (Strawberry) and $y=-365.9\ln(x)+1627.9$ $R^2=0.5321$ (Watermelon). It showed that the relationship between root-knot nematode and organic matter was negative correlation and as the density of root-knot nematode in soil got higher, the amount of soil organic matter was lower. The mean of plant parasitic nematodes was the highest at sandy loam in correlation between density of root-knot nematode and soil textures.

MOLECULAR BIOLOGICAL ANALYSIS FOR THE IDENTIFICATION OF *MELOIDOGYNE* SPECIES IN STRAWBERRY AND ORIENTAL MELON. **Heon-Il, K.[1,2], Y.J. Ko[1,2], G. Eun[1,2], N.S. Park[2], D.G. Kim[3], and I.S. Choi[1,2].** [1]Dept. of Plant Bioscience, Pusan National University, Miryang, 627-706, Korea; [2]Nematode Research Center, Life and Industry Convergence Research Institute, Pusan National University, Miryang, 627-706, Korea; [3]Organic Agriculture Research Institute, Gyeongsangbukdo Agricultural Research & Extension Services, Uiseong, 769-803, Korea.

A survey of root-knot nematodes (*Meloidogyne* spp.) in strawberries and oriental melons in Korea was conducted. Molecular analyses were employed for the identification of *Meloidogyne* spp. DNA sequence analyses were performed on the D3 expansion segment of the 28S gene in the ribosomal DNA in an effort to characterize genetic variations in the three *Meloidogyne* species obtained from Korea. Further, PCR-RFLP, SCAR PCR and RAPD were also utilized to develop methods for the accurate and rapid species identification of the root-knot nematode species. 28S rDNA of 3 root-knot nematode (RKN) sample sets in strawberries (ADSB, HYSB and GCSB) and 2 RKN sample sets in oriental melons (SJOM and GSOM) was cloned and sequenced. 28s rDNA D3 regions of 5 sets were aligned with 7 haplotypes. As a result, 3 sample sets in strawberries showed haplotype 1 and 2 sample sets in oriental melons showed haplotype 5. Two phylogenetic trees

were built based on D3 regions of *Meloidogyne* spp. Three sample sets in strawberries were in the clade of *M. hapla*, and 2 sample sets in oriental melons were in the clade of *M. incognita*, *M. javanica* and *M. arenaria*. COII/lrRNA regions of 5 sample sets were cloned and sequenced. ADSB, HYSB and GCSB had the same 528 bp COII/lrRNA region. However SJOM and GSOM had 2 different 1639 bp COII/lrRNA regions. COII/lrRNA regions, there was one *Hinf*1 restriction site, indicating they were *M. incognita*. RFLP analysis was conducted for SJOM and GSOM with digested with *Hinf*1 restriction enzymes. SJOM and GSOM isolate evidenced fragments of approximately 1300bp and 400bp. This result is shown only in *M. incognita*. RAPD results indicated ADSB and GCSB were *M. hapla*. SCAR experiments showed ADSB and HYSB were *M. hapla*. In conclusion, our results indicated that the 3 sample sets in strawberries were *M. hapla*, and 2 sample sets in oriental melons were *M. incognita*.We results suggest that the most useful molecular method for RKN identification is gene sequence analysis and RFLP of COII/lrRNA region.

THE NEMATICIDAL MODE OF ACTION OF FLUENSULFONE. **Holden-Dye, L., J. Kearn, A. Crisford, E. Ludlow, P.E. Urwin[1], C.J. Lilley[1], and V. O'Connor.** University of Southampton, Centre for Biological Sciences, Bassett Crescent East, University of Southampton, Southampton, SO17 1BJ, UK. [1]Centre for Plant Sciences, School of Biology, University of Leeds, Leeds LS2 9JT, UK.

The new nematicide fluensulfone (Nimitz®) is selectively toxic towards nematodes and thus has reduced potential for environmental damage. We have been investigating the mode of action of fluensulfone using the model organism *Caenorhabditis elegans*, and the cyst nematode *Globodera pallida*. Fluensulfone is nematicidal towards *C. elegans* however relatively high concentrations are required compared to those that are effective in plant parasitic nematodes. Nonetheless, the profile of effects indicates a mode of action that is distinct from other nematicides and anthelmintics (Kearn *et al*., 2014). Its effects on motility, egg-laying and pharyngeal activity suggests pleiotropic actions that could involve distinct pathways or a common mechanism that outputs to different behavioural endpoints. We have been investigating the latter hypothesis and that this may involve a nematode-specific serotonin pathway. We have extended our investigations to plant parasitic nematodes, where fluensulfone is more potent, to further address this question. In *G. pallida*, fluensulfone is an extremely potent inhibitor of hatching of J2s from cysts with a threshold of 0.2 ppm and complete inhibition at 3 ppm. This inhibition is very slow to reverse when fluensulfone is removed from the medium. Fluensulfone also affects stylet thrusting. Over a slower time-course it imparts a concentration-dependent slowly developing metabolic insult in which *G. pallida* J2 transition from impaired motility through to death. Again, this effect is mediated by very low concentrations i.e. 0.2 ppm. The nematicidal characteristics of fluensulfone in *G. pallida* thus involve an impairment of metabolic function. We are currently investigating the possibility that this is triggered through a highly selective interaction with a plant parasitic nematode-specific serotonin pathway.

MODELING ENTOMOPATHGENIC NEMATODE POPULATION DYNAMICS IN AGROECOSYSTEMS. **Hoy, C.** Agroecosystems Management Program, Ohio Agricultural Research and Development Center, 1680 Madison Ave., Wooster, OH 44691.

Biological research on entomopathogenic nematodes has progressed from discovery and description to detailed characterization of physiology and behavior. Excellent progress has been made in designing strategies for the use of cultured entomopathogenic nematodes as a biological insecticide. However, direct observation of entomopathogenic nematodes in their natural habitats is not feasible, and their ecological roles and population dynamics in natural and managed ecosystems must be inferred through sampling at specific locations and times. Simulation models can provide insight into population dynamics, and incorporate known biology to test complex hypotheses about population processes. Progress on modeling nematode population dynamics has lagged behind modeling efforts for other agriculturally important biota. Examples of mathematical modeling in entomology, plant pathology, weed and crop science demonstrate the potential of applying techniques in quantitative ecology to nematology and to entomopathogenic nematodes as biological control agents in particular. Ecological and population dynamics models constructed to date have explored issues surrounding optimization of entomopathogenic nematode application as pesticides and of hypotheses that could explain the observed temporal and spatial variation in field surveys. Future population dynamics modeling could adapt mathematical methods used in other biological systems to describe, explain and visualize the dynamics of entomopathogenic nematodes in both managed and unmanaged ecosystems, and estimate the ecosystem services that these species provide.

METAGENOMIC ANALYSIS OF MICROBIAL COMMUNITIES ASSOCIATED WITH *HETERODERA GLYCINES* IN A SUPPRESSIVE SOIL. **Hu, W.[1,3], S. Chen[1,2], D.A. Samac[2], and X. Liu[3].** [1]University of Minnesota Southern Research and Outreach Center, 35838 120th Street, Waseca, MN 56093; [2]Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108; [3]State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, China.

Suppressive soil harbors potential biological agents controlling plant diseases. However, given the rich and complex suppressive factors, the specific mechanisms of disease suppression have been difficult to identify. Also, the relationships between agricultural practices and suppressive factors are poorly understood. In order to investigate microbial factors involved in suppression of the soybean cyst nematode (SCN, *Heterodera glycines*) in various cropping systems, we conducted a metagenomic analysis of bacterial and fungal communities in bulk soil, rhizosphere soil, and SCN cysts in an SCN-suppressive field

with conventional tillage (CT) and no-tillage (NT) as main treatments, and corn-soybean rotation, soybean monoculture, and soybean monoculture with formaldehyde treatment as sub-treatments. This field has a long history of soybean monoculture, and the main treatments and sub-treatments were maintained for four years before sampling. Bulk soil was sampled at planting and midseason, and cysts were extracted from it. In addition, rhizosphere soil was sampled at midseason. The Illumina Miseq platform was used to sequence the bacterial 16S rRNA V4 region and fungal rRNA ITS1. Using weighted UniFrac distance matrices, bacterial α diversity was enriched by CT compared to NT in all samples while β diversity was not significantly changed by tillage. Formaldehyde reduced bacterial α diversity in cysts and rhizosphere soil and changed community composition in bulk soil and cysts dramatically. Specifically, formaldehyde enriched relative abundance of Proteobacteria in all three types of samples, beta-proteobacteria in bulk soil, and gamma-proteobacteria in cysts. In addition, rotation resulted in greater bacterial diversity than monoculture in rhizosphere soil. Among the three types of samples, bulk soil had the highest bacterial diversity while cysts had the lowest. The fungal community had a different response to tillage and rotation-formaldehyde treatments. Tillage did not affect fungal α diversity or richness. Rotation enriched α diversity but it was reduced in formaldehyde-treated bulk soil and rhizosphere soil. Non-metric multidimensional scaling using Bray-Curtis distance matrices showed that fungal community composition in all samples was affected by tillage, rotation, and formaldehyde. Some fungal taxa, which were frequently isolated from SCN cysts, or have shown potential for control of SCN such as *Pochonia*, *Phoma*, *Cylindrocarpon*, and *Stagonosporopsis*, were slightly reduced in cysts from formaldehyde-treated soil. Bacterial and fungal communities in formaldehyde-treated bulk soil and cysts from formaldehyde-treated soil clustered together using hierarchical clustering with Bray-Curtis distance matrices. This supports our conclusion that formaldehyde had a significant impact on the microbes of suppressive soil. Tillage had minor effects on the soil microflora. Rotation resulted in the highest variation of bacterial and fungal communities in rhizosphere soil. These results support the role of specific microbial communities in suppression of SCN in suppressive soils developed through crop monoculture.

PREDATOR-PREY RELATIONSHIP AS WELL AS THE UNDERLYING MOLECULAR MECHANISM IN THE INTERACTION BETWEEN BACTERIA AND NEMATODES. **Huang, X., K.Q. Zhang, Q.H. Niu, X. Wang, G.H. Li, C.G. Zou, and X.D. Deng.** Laboratory for Conservation and Utilization of Bio-Resources, and Key Laboratory for Microbial Resources of the Ministry of Education, Yunnan University, Kunming, China. 650091.

Nematodes are among the most abundant multi-cellular organisms on the earth, and a complicated micro-ecological system forms between bacteria and nematodes. Some bacteria are demonstrated to have significant toxins against nematodes, and thus are an important group of natural enemies of nematodes. We investigated the molecular and cellular mechanism of interaction between a nematophagous bacterium *Bacillus nematocida* B16 and its nematode hosts and identified a novel mechanism of microbial pathogenesis. The bacterium *B. nematocida* lures nematodes by emitting potent volatile organic compounds that overall are much more attractive than those from dietary bacteria. Following entrapment, the bacteria secrete two proteases to digest intestinal tissues and cause nematode death. Both proteases have broad substrate ranges, including twelve preferentially targeted proteins that play potentially important roles in the structure and function of the intestine. Additionally, it has further been found that the involvement and necessity of ComP-ComA system in *B. nematocida* B16 for its pathogenicity against nematodes. This quorum sensing system controls the synthesis of attractants, production of extracellular degradative enzyme, biosynthesis of secondary metabolites, as well as transporters and so on, most of which are different from that in a saprophytic relative *B. subtilus* 168. Consistently, the mutants in ComP-ComA system displayed the obvious deficiencies in attracting and killing nematodes, due to the absence of attractive signal molecules and the decreased expressions of virulence factors. Another mechanism of bacteria-nematode interaction is through the nematode-trapping fungi. Many bacteria in natural environment are preyed by the bacterivorous nematodes, but our recent study has suggested that those bacteria preyed by nematodes are not wholly passive victims since they can instigate the nematode-trapping fungi to change their life stage from saprophytic to predacious stage. The bacteria produce urea to induce trap formation of a variety of fungi such as *Arthrobotrys oligospora*, and urea-production also favors the trap activities. In a further study of the uptake and transformation of urea in the nematode-trapping fungus *A. oligospora*, we found that urea is finally converted into ammonia and carbon dioxide via the involvement of the transporter Utp79 and the urea lyase Ure1. Between the two catabolized products of urea, ammonia has been revealed to effectively elicit the trap formation. Collectively, our current studies have reflected an interesting co-evolution between bacteria, nematodes, and nematode-trapping fungi.

THE MITOCHONDRIAL GENOME OF THE YAM NEMATODE, *SCUTELLONEMA BRADYS*. **Humphreys-Pereira, D.A.[1], L. Flores-Chaves[2], L. Gómez-Alpizar[3], and A.A. Elling[1].** [1]Department of Plant Pathology, Washington State University, Pullman, WA 99164; [2]Laboratory of Nematology-CIPROC, University of Costa Rica, 2060 San Pedro, Costa Rica, [3]Plant Biotechnology Laboratory, Agronomy Research Center, University of Costa Rica, 2060 San Pedro, Costa Rica.

The yam nematode (*Scutellonema bradys*) is one of the causal agents of dry-rot disease in yams (*Dioscorea* spp.) worldwide, and recently in potato (*Solanum tuberosum*) from Africa. The symptoms include tuber rots, surface cracks, and skin blemishes, and can lead to a reduction of tuber quality and rejection for export. Little is known about the diversity and

origin of *S. bradys*, which might be linked to the origin of yams, its main host. The availability of DNA markers would allow studying the intra-specific variability of *S. bradys*, give insights into its origin and evolutionary history, and improve tools for the molecular identification of the species. In this study, we sequenced the complete mitochondrial (mt) genome of two haplotypes found in two populations of *S. bradys* from Costa Rica. The two haplotypes were designated based on cytochrome b (*cob*) and cytochrome c oxidase subunit 1 (*cox1*) sequences. DNA was extracted from twenty individuals per population (10 females and 10 males). Universal degenerate mt DNA primers were designed from the alignment of 12 protein-coding genes of several plant-parasitic nematodes. The *cob* and *cox1* genes were amplified and sequenced from the 40 individuals. DNA from individuals with the same haplotype was combined and long range PCR was performed with specific and degenerate primers. DNA fragments were cloned and 5-10 plasmid clones per fragment were sequenced. All sequences from the same fragment were identical. Overlapping of the DNA fragments indicated that the *S. bradys* mt genome is circular with a length of ~18 kb. Haplotype 1 was found in 60% of the individuals, and the haplotypes were not skewed towards a specific gender. Pairwise comparison of the protein-coding genes showed divergence levels ranged from 0% (*atp6*) to 5.1% (*nad4L*). Model-based approaches (Bayesian Inference and Maximum likelihood) placed *S. bradys* in a close phylogenetic position to *Heterodera glycines* instead of other migratory endoparasitic nematodes, such as *Pratylenchus vulnus* or *Radopholus similis*.

MITOCHONDRIAL GENOME PLASTICITY WITHIN THE GENUS *MELOIDOGYNE*. **Humphreys-Pereira, D.A. and A.A. Elling.** Department of Plant Pathology, Washington State University, Pullman, WA 99164.

The genus *Meloidogyne* (root-knot nematodes) consists of approximately 100 species and is considered as the most important group of plant-parasitic nematodes. Root-knot nematodes are highly variable and include species with different reproductive modes, climate preferences and host specializations. Recent studies indicate that some species might have originated from intrageneric hybridization events. Phylogenetic and evolutionary analyses of the genus *Meloidogyne* based on complete mitochondrial (mt) DNA genomes have been lacking to date. In this study, the mt genome plasticity within *Meloidogyne* was analyzed by sequencing five mt genomes (*M. arenaria*, *M. chitwoodi*, *M. enterolobii*, *M. incognita* and *M. javanica*). Overlapping DNA fragments indicated that the mt genomes are circular, with a size range from 18.6 to 19.6 kb. The mt genomes contain 12 protein-coding genes (*cox1–3*, *nad1–6*, *nad4L*, *cob* and *atp6*), two ribosomal RNA genes (*rrnS* and *rrnL*) and 22 transfer RNA (*trn*) genes. A comparative analysis between these five mt genomes and the mt genome of *M. graminicola* revealed different gene architectures within the genus. Most of the differences consisted in translocations of *trn* genes and in the position, length and nucleotide composition of non-coding genes. However, there was no specific pattern or link to the natural environments or reproductive modes of the respective *Meloidogyne* spp. The gene order in *M. arenaria*, *M. incognita* and *M. javanica* was identical. *M. enterolobii* was characterized by having three main non-coding regions instead of two as in the other tropical species (*M. arenaria*, *M. incognita* and *M. javanica*). Importantly, the *M. graminicola* mt genome contains extra copies of *trnV* and *trnS2* with high divergence levels. The phylogenetic position of *Meloidogyne* spp. within the phylum Nematoda was determined using model-based methods. Gene architecture and phylogenies based on 12 protein coding genes supported that the migratory endoparasitic nematode *Pratylenchus* is the most recent common ancestor of *Meloidogyne*. Furthermore, the tropical and mitotic parthenogenetic species, *M. arenaria*, *M. enterolobii*, *M. incognita* and *M. javanica* were placed in the same monophyletic group, and the meiotic parthenogenetic species, *M. chitwoodi* and *M. graminicola* in another. Currently, we are analyzing the phylogenetic relationships of *Meloidogyne* when including the 12 protein-coding genes and two *rrn* genes and the effect of single genes or partitioning the data. Sequencing additional *Meloidogyne* mt genomes will enable a high-resolution analysis of the evolutionary history of the genus.

GENETIC DIVERSITY OF THE COLUMBIA ROOT-KNOT NEMATODE, *MELOIDOGYNE CHITWOODI*, IN THE UNITED STATES. **Humphreys-Pereira, D.A., T.L. Peever, and A.A. Elling.** Department of Plant Pathology, Washington State University, Pullman, WA 99164.

The Columbia root-knot nematode, *Meloidogyne chitwoodi* is a major problem in potato-producing regions worldwide and has been designated as quarantine pest in many countries. The wide host range of *M. chitwoodi* makes crop rotation as a control tactic largely ineffective. $R_{Mc1(blb)}$, a resistance gene effective against *M. chitwoodi* has been introgressed into cultivated potato (*Solanum tuberosum*) from the wild *S. bulbocastanum*. However, *M. chitwoodi* pathotypes able to reproduce on potatoes carrying the gene $R_{Mc1(blb)}$ have been found in the western United States. Studying the distribution and genetic diversity of *M. chitwoodi* will provide insights into the geographic origin of this nematode and provide critical information for potato breeding programs. Initially, we sequenced four loci for population studies including two mitochondrial (mt) loci, cytochrome c oxidase subunit 1 (*cox1*) and cytochrome b (*cob*), and two nuclear loci, RNA polymerase II and elongation factor 1 (*EF1*) from six *M. chitwoodi* populations. The *cob* and *EF1* markers had sufficient diversity for population studies and currently are being tested on 25 *M. chitwoodi* populations from the western United States. The *cob* locus was amplified and sequenced from 540 single J2 individuals. Four segregating sites were observed in ~900 bp and 3 mt haplotypes were designated based on these differences. All nucleotide substitutions were transversions (A ↔ T) with two changes in the third codon position and one change each of the first and second codon positions, respectively. One of four substitutions was synonymous and three of four substitutions were non-synonymous. Pairwise comparisons revealed 0.22%

divergence between haplotype 1 and haplotypes 2 and 3, whereas haplotypes 2 and 3 diverged by 0.45%. Haplotype 1 represented 71% (382) of individuals, followed by haplotype 2 with 19% (100) and haplotype 3 with 11% (58). All three haplotypes were present in a population from Quincy, Washington, the same location from which *M. chitwoodi* was first described in the 1980s. Four populations contained two haplotypes and the remaining populations (20) had unique haplotypes. Bulk DNA was extracted from 100 individuals per population to test *EF1*, the second marker. Partial sequences of the nuclear gene *EF1* were amplified from bulk DNA and cloned. Fifteen plasmid clones per population were sequenced. A preliminary alignment of sequences from 12 populations showed 26 segregating sites and high values for nucleotide diversity (0.0094 to 0.0183) and haplotype diversity (0.59 to 0.89) were observed in the 12 populations.

SURVEY OF PHYTOPARASITIC NEMATODES ASSOCIATED WITH SOME CROP PLANTS IN NORTHERN EGYPT. **Ibrahim, I.K.A.[1] and Z.A. Handoo[2].** [1]Department of Plant Pathology, Faculty of Agriculture, Alexandria University, Alexandria, Egypt; [2]Nematology Laboratory, USDA, ARS, Beltsville, MD 20705.

Information concerning the occurrence and distribution of phytoparasitic nematodes in Egypt is very important for agricultural production. A nematode survey was conducted in northern Egypt to identify the genera and species of phytoparasitic nematodes associated with some crop plants. A total of 240 soil and root samples was collected from the rhizosphere of the surveyed plants and then processed for nematode extraction and identification. Twenty-two genera of phytoparasitic nematodes were detected in the collected soil and root samples. In soil samples from Alexandria governorate, the sugar beet cyst nematode *Heterodera schachtii* and the root-knot nematodes *Meloidogyne incognita* and *M. javanica* were very common on sugar beet. *Helicotylenchus pseudorobustus*, *M. incognita, Pratylenchus* sp., *Rotylenchulus reniformis* and *Xiphinema* sp. were observed in spearmint soil samples. The dagger nematode *Xiphinema rivesi* was found in orange soil samples from EL-Nobarria, EL-Behera governorate. In lantana soil samples from EL-Giza governorate, *Aglenchus geraerti*, *Bitylenchus ventrosignatus*, *Coslenchus capsici*, *Helicotylenchus indicus*, and *Malenchus bryanti* were identified for the first time in Egypt. Survey results revealed new host plant records for most of the identified nematode species in Egypt.

EFFECTS OF THE CYST NEMATODE *GLOBODERA ELLINGTONAE* ON POTATO. **Ingham, R.[1], W.S. Phillips[2], A. Peetz[2], N.M. Wade[1], and I.A. Zasada[2].** [1]Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331-2902; [2]USDA ARS Horticultural Crops Research Laboratory, 3420 NW Orchard Ave., Corvallis, OR 97330.

A new cyst nematode species, *Globodera ellingtonae*, was described from Powell Butte, Oregon and two grower fields in Idaho, U.S.A. in 2012. Initial studies indicated a close phylogenetic relationship of this nematode to the potato cyst nematodes *G. pallida* and *G. rostochiensis*, and demonstrated that *G. ellingtonae* reproduces readily on potato (*Solanum tuberosum*). Therefore the potential for *G. ellingtonae* to reduce yield of potato is of great concern. To evaluate the pathogenic effects of *G. ellingtonae* on potato, five field trials over a three-year period were conducted. In three trials (2012, 2013, 2014), single hills of potato 'Russet Burbank' were planted into soil infested with different initial densities (Pi) of *G. ellingtonae* (0, 5, 10, 20, 40, or 80 eggs/g soil) at the Central Oregon Agriculture Research Center farm at Powell Butte, OR. In 2013, the trial was repeated with the red potato variety, Désireé. In another trial (2014), five additional potato varieties varying in maturity lengths were either inoculated (80 eggs/g soil) or not with *G. ellingtonae*. All trials were planted in a randomized block design with 7 or 8 replications on a 76 cm in-row spacing between plants. At harvest, tops were removed, dried and weighed (2013 and 2014), and tubers were dug by hand and weighed. Soil samples were taken from beneath each plant; cysts were extracted and crushed to determine the eggs per g soil (Pf). Pf densites for Pi densities of 5, 10, 20, 40, and 80 eggs/g soil averaged 104, 121, 177, 234, and 229 eggs/g soil, respectively, for the four trials with variable Pi. This suggests that some limit in nematode reproduction may have been reached between 40 and 80 eggs/g soil. Only one of the trials (2013) conducted with increasing levels of Pi, resulted in a significant negative correlation between Pi and yield of Russet Burbank. Combining data from the three years of Russet Burbank trials in a multiple linear regression model indicated a significant effect of Pi on tuber yield. Based on the linear regression model of tuber yield on log(Pi) with a single slope for the three Russet Burbank trials, 11.3 to 17.0% yield loss is predicted at a Pi of 40 eggs/g soil and 13.5 to 20.2% yield loss is predicted at a Pi of 80 eggs/g soil when tuber yields at Pi of 0 eggs/g soil are 1,829 to 2,744 g/plant. None of the potato varieties inoculated with 80 *G. ellingtonae* eggs/g soil had significantly reduced yields compared to non-inoculated plants. Care should be taken in extrapolating the results from this single field site to probable effects of *G. ellingtonae* on potato in other environments.

FUNCTIONAL GENETICS OF A DEVELOPMENTAL SWITCH IN THE DIMORPHIC NEMATODE *PRISTIONCHUS PACIFICUS*. **Ivers, N.A. and E.J. Ragsdale.** Department of Biology, Indiana University, 915 E 3rd Street, Bloomington, IN 47405.

Developmental plasticity, especially polyphenism, is increasingly thought to foster rapid evolution and diversification, besides facilitating adaptation in unstable or intermittent environments. However, the genetic regulation of plasticity has only recently begun to be understood. Here we use the laboratory model *Pristionchus pacificus* (Diplogastridae), which is dimorphic in its feeding structures, to unravel the genetic pathway for a polyphenism switch. In response to environmental cues such as the availability of bacterial food, *P. pacificus* develop into adult forms that differ in the number and size of their

teeth: its stenostomatous (St) morph has a simple, narrow stoma (mouth) suited for feeding on microbes, whereas an alternative (eurystomatous, Eu) morph has large, opposable teeth that allow predatory feeding on other nematodes. The development of these alternative forms is regulated in part by a recently discovered switch gene, *eud-1* (Eu-form-defective), the expression of which promotes the Eu mouth form. However, the functional context of this switch gene has yet to be described. To further understand the genetic regulation of the polyphenism, we conducted a forward genetic screen for genes acting downstream of *eud-1*, specifically suppressors of a *eud-1* null mutant. A screen of 10,300 mutant genomes resulted in the recovery of 9 *seu* (suppressor-of-Eud) mutant alleles belonging to at least four complementation groups. Among these mutants are both X-linked and autosomal loci, as well as both dominant and recessive alleles, suggesting the potential to reconstruct a pathway for the polyphenism switch. Genetic mapping and genomic re-sequencing of these mutants will help to identify the genes discovered. Thus, an unbiased search for causal factors underlying the diplogastrid mouth dimorphism in this nematode system has demonstrated the potential to discover the genetic elements that regulate an environmentally responsive trait.

GLAND MINING AND EFFECTOR CHARACTERIZATION FROM *HETERODERA* CYST NEMATODES. **Juvale, P.S.[1], G.V. Pogorelko[1], T.R. Maier[1], M.G. Mitchum[2], E.L. Davis[3], and T.J. Baum[1].** [1]Department of Plant Pathology and Microbiology, 351 Bessey Hall, Iowa State University, Ames, IA 50011; [2]Division of Plant Sciences, 371H Life Sciences Center, University of Missouri, Columbia, MO 65211; [3]Campus Box 7616, 2510 Thomas Hall, North Carolina State University, Raleigh, NC 27695.

The soybean cyst nematode (*Heterodera glycines*, SCN) is regarded as the most damaging pathogen of soybeans. Developing soybean cultivars with novel, long-lasting resistance to SCN is a major priority. In order to pursue this goal, generating a deep knowledge base about soybean-SCN interactions during the infection is essential. SCN is a highly evolved obligatory sedentary pathogen that induces massive cellular reprogramming at the site of infection to establish the syncytium feeding site. It is generally accepted that a mix of effector proteins delivered into the soybean tissue by the nematode is responsible for the comprehensive reprogramming of the host tissue as well as evading host defense responses. Our research focusses on identifying and characterizing SCN effectors to uncover the molecular mechanisms that allow SCN infection and parasitism.

In our most recent efforts to identify novel effectors, we have developed a method to specifically stain and isolate nematode esophageal gland cells, which are the major sources of effectors. RNA is extracted from these purified gland cells and processed for high throughput sequencing. Subsequent bioinformatic analyses allow the identification of novel effectors. Currently, sequencing projects are underway from multiple nematode species. For the molecular characterization of identified effectors we are using molecular and biochemical techniques and have extensively used the *Heterodera schachtii* (sugarbeet cyst nematode; BCN)-Arabidopsis model pathosystem to make fast gains. One such effector being characterized appears to be involved in the manipulation of the defense pathway. Transgenic Arabidopsis plants expressing this effector gene show altered susceptibility to multiple pathogens, and protein-protein interaction studies have shown that this effector specifically interacts with and relocalizes a vacuolar protease with known defense functions. In another example, transgenic Arabidopsis lines expressing an effector are hyper susceptible to the nematode. Our data indicate that this effector interferes with a unique signal transduction pathway by specifically interacting with a stress induced protein kinase.

By conducting characterizations of a number of cyst nematode effectors our work is generating an understanding of the physiology of parasitism. Ultimately, this understanding will allow the development of novel management options for this and other pathogens.

HETERODERA SCHACHTII POPULATION DENSITY AND DAMAGE RELATIONSHIPS ON OILSEED RAPE (OSR; *BRASSICA NAPUS* L.) IN THE UNITED KINGDOM. **Kakaire, S., I.G. Grove, and P.P.J. Haydock.** Nematology and Entomology Group, Dept. of Crop and Environment Sciences, Harper Adams University, Newport, Shropshire, TF10 8NB, UK.

The increasing OSR hectarage in the United Kingdom and the rising soil temperatures are potential risk factors for OSR damage and yield loss as a result of cyst nematodes. Outdoor and polytunnel pot experiments were conducted in 2010/2011 to investigate the number of generations completed by *Heterodera schachtii* on two winter OSR cultivars (cvs; Flash and Castille) and two spring cvs (Belinda and Heros) during the growing season, as well as the response of five winter cvs (Castille, Flash, Catana, DK Carbernet, and ES Astrid) to *H. schachtii* infection, respectively. A randomized complete block design with six replicates was used in both experiments. In the outdoor pot experiment, soil and roots from five plants per treatment were harvested monthly and processed by standard methods for nematode extraction. The total numbers of the different *H. schachtii* developmental stages in 2 ml aliquots of the root samples were counted under a stereo microscope at ×60. Two replicate sub-samples of the pot soil, each 200 g, were extracted by a modified Baermann funnel method, and the second stage juveniles (J2) in 2 ml aliquots were counted under a stereo microscope at ×60. A generation was deemed completed when large numbers of J2 (J2 peaks) were recovered from the soil during monthly soil samplings. In the

polytunnel experiment, *H. schachtii*-infested soil was diluted with sterile kettering loam, which also served as the control, to produce five initial population densities of 2, 4, 8, 16, and 32 (eggs g soil)$^{-1}$. The *H. schachtii* damage threshold on the five winter OSR cultivars and the effect on plant growth and yield were assessed 12 weeks after planting. The final population densities and nematode multiplication rates on each cultivar were determined after extraction of pot soil by fluidizing column. The data were analyzed using dose-response ANOVA in Genstat release 13.1; whilst pair-wise comparisons were conducted using Tukey's multiple range test to identify significant differences between treatment means at $P < 0.05$. The relationship between the initial, final population densities and multiplication rates were determined using linear and group regression analyses. At least one generation was completed on cv. Castille whilst two generations were completed on cvs Flash, Belinda and Heros during the OSR growing season. Cultivar Flash was more susceptible to *H. schachtii* infection than cv. Castille, whilst cv. Belinda was more susceptible than cv. Heros. Cultivars Flash and DK Cabernet were more susceptible to *H. schachtii* damage than cvs ES Astrid, Castille and Catana, respectively. However, no major plant growth differences were observed between the cvs. Nematode multiplication rates were generally low and the damage threshold was between 2 and 8 (eggs g soil)$^{-1}$ on all the cvs investigated.

ALLODIPLOGASTER SPECIES (DIPLOGASTRIDAE) FROM SOIL-DWELLING BEES IN THE EASTERN UNITED STATES. **Kanzaki, N.[1], R.M. Giblin-Davis[1], and E.J. Ragsdale[2].** [1]Fort Lauderdale Research and Education Center, University of Florida/IFAS, 3205 College Ave, Davie, Florida 33314-7799; [2]Department of Biology, Indiana University, 915 E. 3rd Street, Bloomington, IN 47405.

Two commensal associates of bees, *Allodiplogaster* sp. (RGD227) from the Dufour's gland of a cellophane bee (*Colletes thoracicus*) from Maryland and *Allodiplogaster* sp. (RGD228) from the abdominal glands of an andrenid bee (*Andrena alleghaniensis*) from New York are morphologically and phylogenetically characterized. Both species were collected as dauers from their respective hosts and cultured on bacteria on tryptic soy broth or NGM agar. *Allodiplogaster* species RGD227 and RGD228 are morphologically closer to each other than to other species of *Allodiplogaster*, which was recently revised to include 37 valid species. However, the two new species are distinguished by reproductive isolation, manubrium shape of the spicules in males, host associations, and molecular characters, the latter in sequences of the near-full length small subunit rRNA gene, D2-D3 expansion segments of the large subunit rRNA gene, and partial mitochondrial COI. Morphological characterization was supplemented by scanning electron microscopy which revealed furcation of both v5 and v6 male genital papillae, consistent with previous reports for species of the *henrichae* group of *Allodiplogaster*. Congruent with this finding, molecular phylogenetic analysis based on SSU sequences suggests that these two new species form a well-supported clade with other *henrichae*-group species. Finally, to test our characterizations of the new species, we performed mating studies. Crosses of *Allodiplogaster* sp. RGD228 to *Allodiplogaster* sp. RS1982, isolated from cockchafers in Europe from Dr. Ralf Sommer (Max Planck Institute for Developmental Biology) produced fertile hybrids and were thus shown to be biologically conspecific, whereas both of these strains were reproductively isolated from *Allodiplogaster* sp. RGD227.

RUEHMAPHELENCHUS SP. (APHELENCHOIDIDAE) FROM AN AMBROSIA BEETLE FROM SOUTH FLORIDA. **Kanzaki, N.[1], R.M. Giblin-Davis[1], R. Gonzalez[1], R. Duncan[2], and D. Carrillo[2].** [1]Fort Lauderdale Research and Education Center, University of Florida/IFAS, 3205 College Ave, Davie, Florida 33314-7799; [2]Tropical Research and Education Center, University of Florida/IFAS, 18905 SW 280 Street, Homestead, FL 33301.

During a survey of nematode associates of ambrosia beetles from dead and dying red bay and avocado trees affected by the laurel wilt epidemic in southern Florida, a *Ruehmaphelenchus* species was isolated from the non-native ambrosia beetle, *Xylosandrus crassiusculus*. The nematode was examined for its species status based on morphological and phylogenetic characters, and considered an undescribed species. The new species is characterized by its possession of an oral disc at the stomatal opening, three lines in the lateral field, male spicule with clear dorsal and ventral limbs connected by elongated triangular cuticle, thin membrane-like tissue and cuticular bridge-like structure, conical tail with pointed tip of males and conical tail with digitate mucro of females. The new species is typologically considered a ''cryptic species'' with a complex of four previously described species, i.e., *R. asiaticus*, *R. digitulus*, *R. thailandae* and *R. sirisus*, and can be distinguished from them only by minor morphological differences in male tail characters, *i.e.*, spicule morphology, position of genital papillae and tail tip shape, and morphometric values. However, the new species is phylogenetically unique, *i.e.*, the phylogenetic analysis based on near-full length small subunit (SSU) rRNA gene, D2-D3 expansion segments of the large subunit rRNA (D2-D3) genes of ribosomal RNA suggested that the species is the basal taxon of the *Ruehmaphelenchus* clade, and close to other *Bursaphelenchus* species. Further, in addition to SSU and D2-D3, the molecular sequences of the internal transcribed spacer region, D1 expansion segment of LSU and partial mitochondrial COI genes are ascribed for the species (isolate)-specific barcode. Because the nematode was isolated from an invasive species, the geographical origin of the species has not been clarified. Wide-ranging geographical surveys of ambrosia beetle-associated nematodes are necessary because of the potential spread of these beetles and their associated nematodes.

MELOIDOGYNE SPP. REPORTED FROM ARKANSAS: PAST AND PRESENT. **Khanal, C.[1], R.T. Robbins[2], E.C. McGawley[1], and C. Overstreet[1].** [1]Department of Plant Pathology and Crop Physiology, Louisiana State University, Baton Rouge, LA, 70803; [2]Department of Plant Pathology, University of Arkansas, Fayetteville, AR 72701.

Five species of *Meloidogyne* (*M. incognita, M. arenaria, M. javanica, M. hapla* and *M. graminis)* have previously been reported from Arkansas in studies conducted between 1964 and 1994. These identifications were based on morphological characteristics and host differential assays. Polymerase chain reaction (PCR) and sequencing methodology were employed in research reported herein to identify current species of *Meloidogyne* endemic in Arkansas in 2013-2014. A total of 106 soil and root samples, representing 36 of the 75 counties, were collected from a wide range of crop and grass species. Samples were processed to obtain root-knot nematodes. PCR was performed using universal primers to amplify mitochondrial DNA (mtDNA) genes. DNA sequencing data confirmed the presence of *M. incognita, M. haplanaria, M. marylandi, M. hapla, M. arenaria*, and *M. partityla*. Minimal intraspecific variation was observed between the species from different samples and no intraspecific variation was found for the species within the same sample. *Meloidogyne incognita* was the most abundant species and the only one found parasitizing soybean. Unlike previous reports from Arkansas, *M. javanica* and *M. graminis* were not found. This updated information regarding the presence of *Meloidogyne* species in Arkansas benefits farmers and personnel in the state involved in crop management.

COMPARATIVE STUDY OF THE COLONIZATION AND DEVELOPMENT OF *GLOBODERA PALLIDA* IN THE ROOTS OF *SOLANUM TUBEROSUM* AND *SOLANUM SISYMBRIIFOLIUM*. **Rinu, K. and L.M. Dandurand.** Plant, Soil, and Entomological Sciences Department, University of Idaho, Moscow, ID, 83844.

Globodera pallida (pale cyst nematode, PCN) is a major pest in potato growing areas despite existing control measures. Due to the negative impact of various chemicals used as nematicides, developing alternative economically feasible strategies has been a major research focus in recent years. *Solanum sisymbriifolium* (litchi tomato) has potential as a trap crop for PCN, by stimulating egg hatch without allowing nematode reproduction. Previously, we observed that development of *G. pallida* was arrested in *S. sisymbriifolium* roots. Fewer J2s were found in *S. sisymbriifolium* roots, compared to roots of *S. tuberosum* (potato), four weeks post-infection. In the present study, a non-destructive imaging technique was used to compare the infection timeline of *G. pallida* in *S. tuberosum* and *S. sisymbriifolium* roots. J2 juvenile nematodes were pre-stained with the live fluorescent stain, PKH-26, and the plants were planted in microscopy rhizosphere chambers (micro-ROC). These chambers provide a direct root observation area, in which the plant is grown between the coverslip and a thin nylon mesh, and the soil is placed behind the nylon mesh. Observation was done under an inverted fluorescence microscope (Leica, DMI 3000B), and nematode development was recorded over time. On 2 day post-infection (DPI), J2s were found in roots of both plant species. J3s were found 8 days DPI in *S. tuberosum* but not in *S. sisymbriifolium*. However, dead J2s were found in *S. sisymbriifolium* roots after 4 days DPI, and no further nematode development was observed. Fluorescence microscopic observations showed an apparent hypersensitive response in infected root cells of *S. sisymbriifolium*. Understanding the time line of development of PCN in *S. sisymbriifolium* will be important to understand the molecular mechanisms of the defense response and the successful utilization of this potential trap crop in the field.

REVELATION *HETERODERA SCHACHTTII* CYST DISSEMINATION IN HIGHLAND CHINASE CABBAGE FIELDS OF KOREA: LEADS TO INFECTION TO NEW AREA. **Kwon, O-G., J-H. Shin, F. Md. Kabir, and D-W. Lee.** Department of Ecological Science, Kyungpook National University, Sangju, Gyeongbuk 742-711. Republic of Korea.

It has been recently reported that the sugarbeet cyst nematode, *Heterodera schachtii* could cause severe damages in Chinese cabbage, especially in high land vegetables cultivation areas such as Jeongseon, Samcheok and Taebaek in South Korea. Field studies were conducted in year 2014-2015 to reveal the possibility of disseminating cysts through work shoes, work vehicles, and surface runoff of water in rainy conditions in an infected highland Chinese cabbage field. When workers moved around for 10 minutes in Chinese cabbage field infected by the sugarbeet cyst nematode, 13 or 1 healthy cysts were found under worker's shoes in Jeongseon or Samcheok areas, respectively. These results indicate that work shoes play a vital role for carrying cysts to the uninfected field. After driving on a distance of 60 meters to the road close to the sugarbeet cyst nematode infected field, the soil attached to the vehicle's tires contains 1 healthy cyst with 85 eggs and 19 juveniles. These results also suggest that just moving to another field after working by car in an infected field could cause spreading the cysts to the new field in a similar way as those carried by worker's work shoes. Under irrigation conditions of 2, 4 and 8 liters of water, the numbers of healthy cysts in soils from the bottom of shoes examined for two workers (75 and 100kg of body weights) were 48, 88, and 113 in 75kg of worker, and 39, 56, and 76 in 100 kg of worker, respectively in Jeongseon area. Under rainy conditions supplemented by irrigation of 10L of water to ridges, 207 and 85 healthy cysts were found at the edges of 5 m-long ridges in Jeongseon and Samcheok areas, respectively, which clearly indicated that dissemination of cysts mainly caused by surface runoff during rainy condition. Taken together, our results imply that personal sanitizer is one of the most important factors for blocking dispersal of the sugarbeet cyst nematode in field.

ANAEROBIC SOIL DISINFESTATION (ASD) COMBINED WITH SOIL SOLARIZATION FOR ROOT-KNOT NEMATODE CONTROL IN VEGETABLE AND ORNAMENTAL CROPS IN FLORIDA. **Kokalis-Burelle, N.[1], J. Hong[1], D.M. Butler[2], and E.N. Rosskopf[1].** [1]USDA-ARS, U.S. Horticultural Research Lab, Ft. Pierce, FL 34945; [2]University of Tennessee, Knoxville, TN 37996.

Anaerobic soil disinfestation (ASD) combined with soil solarization continues to be evaluated for management of plant-parasitic nematodes in vegetable and ornamental crops in Florida. ASD combines organic amendments and soil saturation to stimulate microbial activity and create anaerobic conditions in soil covered with polyethylene mulch. Research has focused on root-knot (*Meloidogyne* spp.) nematode control, and effects on free-living nematodes. Field trials were conducted to determine composition and levels of inputs necessary to control root-knot nematodes (RKN). In initial double-cropped pepper-eggplant trials, *M. incognita* juvenile (J2) numbers were low through the first season following ASD. By the second eggplant crop, solarization (no amendments or water) averaged greater than 200 nematode J2/100 cm^3 soil compared to 10/100 cm^3 in ASD with amendments and irrigated with 5-10 cm of water. Application of molasses alone or with composted broiler litter (CBL) combined with irrigation caused reductions in RKN J2 in soil and eggplant roots in the second season compared to soil solarization without amendments or irrigation. Root galling was greatly reduced in treatments containing molasses compared with solarization alone. Free-living nematodes in soil increased with application of CBL. A similar increase in free-living nematodes occurred with ASD using molasses and CBL with 5 cm of water in strawberry production. In cut flowers, RKN management with ASD was highly dependent on host susceptibility. In three cut flower crops, ASD with molasses, CBL, and 5-cm of water under clear polyethylene resulted in yields equivalent to methyl bromide, but did not provide season-long RKN control on snapdragon, which is highly susceptible to RKN. Recent studies indicate totally impermeable film can be substituted for solarization film, reducing plastic use and simplifying ASD application. ASD has been successfully trialed in tomato, cucumber, bell pepper, eggplant, strawberry, and flower crops and can be customized for production system and local waste product inputs.

THE USE OF NEXT-GENERATION DNA SEQUENCING TO FACILITATE THE GENETIC ANALYSIS OF *HETERODERA GLYCINES*. **Lambert, K.[1], S. Bekal[2], and L. Domier[1].** [1]Department of Crop Sciences, University of Illinois, Urbana, IL 61801; [2]Department of Agricultural and Biological Engineering, University of Illinois, Urbana, IL 61801.

Genetic map-based cloning of genes controlling important nematode phenotypes has been a successful approach in model nematodes. However, this strategy has not been widely employed in sexually reproducing plant parasitic nematodes due to a lack of genomic sequence information. In the past, the cost of DNA sequencing was the primary barrier for plant parasitic nematode genome studies. Today, emerging next-generation DNA sequencing platforms have made it possible to collect vast amounts of DNA sequence for relatively low cost. This revolution in genome sequencing has enabled recalcitrant plant parasitic nematode genomes to be sequenced and the data used for genetic analysis. One such nematode species is *Heterodera glycines*, the soybean cyst nematode (SCN). In this project, the SCN genome and transcriptome were sequenced using three next generation sequencing platforms, 454, SOLiD and Illumina. The goals of this project were to identify expressed SCN genes and to find single-nucleotide polymorphisms (SNPs) that could be used to generate an SCN genetic linkage map for map-based cloning of nematode genes. High-throughput SNP assays enabled both the production of a marker-dense SCN genetic linkage map, but also allelic imbalance studies to identify SNPs linked to important SCN phenotypes. In this initial study, SNPs were identified that were associated with the nematode's ability to reproduce on SCN resistant soybean plants (virulence genes). These virulence associated SNPs were found to cluster in three regions of the SCN genetic map. Expressed SCN genes that were near the virulence-associated SNPs were evaluated and candidate virulence genes were identified. A detailed characterization of one candidate virulence gene indicated it encoded a new effector protein that appears to modulate the same plant pathways as a known SCN resistance gene. Overall, the ability to rapidly collect nematode DNA sequence has now enabled a detailed genetic analysis of SCN for any measurable phenotype. The capacity to identify the underlying genes controlling SCN virulence, aggressiveness, host-range or even susceptibility to diseases will aid in the development of more efficient approaches to manage plant parasitic nematodes.

HATCH STIMULATION AND HOST STATUS OF TOBACCO (*NICOTIANA TABACUM*), EASTERN BLACK NIGHTSHADE (*SOLANUM PTYCHANTHUM*), AND STICKY NIGHTSHADE (*SOLANUM SISYMBRIIFOLIUM*) TO THE TOBACCO CYST NEMATODE, *GLOBODERA TABACUM*. **LaMondia, J.A.** The Connecticut Agricultural Experiment Station Valley Laboratory, PO Box 248, Windsor, CT 06095.

The influence of broadleaf cigar wrapper tobacco (*Nicotiana tabacum*), eastern black nightshade (*Solanum ptychanthum*), and sticky nightshade (*Solanum sisymbriifolium*) on egg hatch and subsequent development of the tobacco cyst nematode, *Globodera tabacum*, was investigated. Root diffusates were prepared from 2 g of root of four-week-old plants soaked in 100 ml of distilled water for 2.5 hours, filtered and frozen. *Solanum ptychanthum* root diffusates stimulated juvenile hatching from eggs in cysts over 4 weeks more than root diffusates of *S. sisymbriifolium* or *N. tabacum*. Tobacco increased hatch by four times compared to water alone; *S. sisymbriifolium* stimulated twice and *S. ptychanthum* three times the hatch of that for

N. tabacum. *G. tabacum* juveniles were observed in stained roots of both *N. tabacum* and *S. sisymbriifolium* and development to adult females occurred within four weeks in tobacco but not *S. sisymbriifolium*. Cysts were extracted from roots and soil in pots that had been planted to *N. tabacum* or *S. sisymbriifolium* for 12 weeks and cysts crushed to count encysted juveniles. Final population densities were 324 *G. tabacum* J2 per 100 cm^3 soil for tobacco and 4.5 *G. tabacum* J2 per 100 cm^3 soil for *S. sisymbriifolium*. Sticky nightshade, *Solanum sisymbriifolium*, stimulates tobacco cyst nematode hatch better than tobacco but unlike eastern black nightshade, does not allow significant reproduction in roots, indicating that it may be an effective trap crop for management of *G. tabacum*. In addition, *G. tabacum* may be useful as a substitute model for the quarantined pathogen *Globodera pallida* for trap cropping with *S. sisymbriifolium* under field conditions.

OUTBREAK OF SUGAR BEET CYST NEMATODE IN KOREA. **Lee, J-K.[1], S.J. Kim[1], B.Y. Park[2], and H.R. Ko[1].** [1]Crop protection Division, National Academy of Agricultural Science, RDA, Jeonju, 560-500, Republic of Korea; [2]Technology Cooperation Bureau, RDA, Jeonju 560-500, Republic of Korea.

During the survey of nematodes in Chinese cabbage in 2011, we found damaged Chinese cabbages in Gangwon province, Korea. Symptoms are yellowing, wilting, stunted growth, and poor standing. White to pale yellow females and brown cysts were found attached on the roots. Further identification using morphometric characters and gene sequencing analysis using the internal transcribed spacer 1 (ITS1) region of ribosomal DNA (rDNA) confirmed it as a *H. schachtii*. Fields were surveyed to determine the distribution and population densities of *H. schachtii* from 2011 to 2014. So far, *H. schachtii* was found only within the highland Chinese cabbage growing areas of Gangwon province. Its population, however, increased significantly in 2014 compared to 2011. Various control practices are under study and white mustard as a trap crop and non-host plants as a rotation crop seems promising.

EVALUATION OF ROOT-KNOT NEMATODE RESISTANCE IN SOYBEAN CULTIVARS IN HEILONGJIANG PROVINCE IN CHINA. **Li, C.[1], C. Hua[1], Y. Mao[1], C. Zhou[2], Y. Hu[1], Z. Tian[2], and C. Wang[1].** [1]Key Laboratory of Mollisols Agroecology, Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences, Harbin 150081, China; [2]Daqing Branch, Heilongjiang Academy of Agricultural Sciences, Daqing 163000, China.

Heilongjiang Province is the largest production area of soybean (*Glycine max*) in China. Soybean cyst nematode (SCN) have been an economically important pathogen in northern China. A series of commercial cultivars resistant to SCN race 3, the dominant SCN race in northern China, were released and have played a key role in controlling SCN race 3. Root-knot nematodes (*Meloidogyne* spp.) were reported to infect soybean in central China, but not in northern China. Root-knot nematodes were found only under protected cultivation of vegetables in northern China. Recent years, root-knot nematodes has been spreading quickly with the increasing protected cultivation of vegetables due to huge demand of vegetables in northern China. However, no knowledge was available about the response of local soybean cultivars to RKN resistance. In order to investigate resistance sources to RKNs, 12 varieties originally resistant to SCN race 3 and 5 cultivars susceptible to SCN race 3 were inoculated with both the southern root-knot nematode (*Meloidogyne incognita*) and the northern root-knot nematode (*M. hapla*) under controlled greenhouse condition. The galling index (1-10 scale) and nematode reproduction were used to evaluate reaction to RKN at 40 d after inoculation for *M. incognita* and 45 d for *M. hapla*. The results from two replicates indicate that 3 out of 12 SCN resistant varieties (Kangxian 10, Kangxian 13 and 09-138) and 2 out of 5 SCN-susceptible cultivars (Suinong 14 and Hefeng 25) showed both a lower galling index (0-2.8) and less egg production (<1000 eggs per gram root, EGR) for *M. incognita* than other varieties. Two out of 17 tested cultivars (Kangxian 6 and Suinong 14) showed a lower galling index (< 2) and less than 2000 EGR for nematode production. Among 17 tested cultivars, the range of root galling index caused by *M. incognita* was 0-6.6 and the range of egg numbers was 172-22,461 eggs per gram root (EGR) for nematode reproduction in one replicate. The range of root galling index caused by *M. hapla* was 0.8-6 and the range of egg numbers was 1310-25,847 EGR for nematode reproduction in one replicate. These resistant or tolerate varieties lines would be valuable breeding sources for RKN resistance.

INFLUENCE OF ROOT EXUDATES ON ATTACHMENT OF *PASTEURIA PENETRANS* TO *MELOIDOGYNE ARENARIA*. **Liu, C.[1], P. Timper[1], T.M. Mengistu[2], and S. Joseph[2].** [1]USDA ARS, P.O. Box 748, Tifton, GA 31793; [2]Entomology and Nematology Dept., University of Florida, Gainesville, FL 32611.

We hypothesized that root exudates would influence the spore attachment of *Pasteuria penetrans* to root-knot nematodes (*Meloidogyne arenaria*). An experiment was carried out using a factorial arrangement of two single spore (SS) lines cultured from *P. penetrans* and three single egg mass (SEM) lines cultured from *M. arenaria* to test the influence of root exudates on spore attachment to second-stage juveniles (J2). The root exudates were obtained by placing three 1-month-old eggplant seedlings in a foil-covered 100ml beaker containing 60 ml of water to submerge the roots and incubating in a greenhouse for 24 h. The J2 from each SEM line were incubated in root exudates for 6 h before 10^5 spores of *P. penetrans* were added to the solution and incubated for an additional for 6 h. The control included J2 incubated in sterilized water instead of root exudates. The experiment was conducted twice (Trial 1 and

Trial 2). Compared to the control, exposure to root exudates reduced (P<0.0001) attachment of both SS lines to SEM14 and reduced (P=0.018) attachment of one SS line to SEM3 in Trial 1. Attachment of SS16 to SEM14 was reduced by 76% and attachment of SS25 to SEM3 and SEM14 was reduced by 54% and 85%, respectively. In Trial 2, exposure to root exudates reduced (P<0.0001) spore attachment in all three SEM lines to both SS lines compared to the control. Spore attachment was reduced by 71%, and 69% for SS16 and SS25, respectively. Differences between the two trials may be due to the method of J2 harvest. In Trial 1, J2 were obtained from roots incubated in a mist chamber where they were pre-exposed to root exudates. However, in Trial 2, the J2 were obtained from eggs that were extracted from roots and incubated in hatching dishes. When J2 of *Meloidogyne* spp. approach roots, their surface coat may be altered, which in turn may reduce their susceptibility to *P. penetrans*.

HOW FILARIAL NEMATODE GENOMES HAVE ACCELERATED THE DISCOVERY OF VACCINE AND DIAGNOSTIC BIOMARKER CANDIDATES. **Makepeace, B.L.[1], A.H. Buck[2], C.K.S. Carlow[3], S.A. Babayan[4], V.N. Tanya[5], D.W. Taylor[1], and M.L. Blaxter[2].** [1]Institute of Infection & Global Health, University of Liverpool, Liverpool L3 5RF, UK; [2]Centre for Immunity, Infection and Evolution, University of Edinburgh, Edinburgh EH9 3JT, UK; [3]Division of Genome Biology, New England Biolabs, Ipswich, MA 01938; [4]Institute of Biodiversity, Animal Health & Comparative Medicine, University of Glasgow, Glasgow G12 8QQ, UK; [5]Cameroon Academy of Sciences, Yaoundé BP 1457, Cameroon.

Filarial nematodes are a highly specialised group of vector-borne parasites of terrestrial vertebrates. They are responsible for two devastating neglected tropical diseases, lymphatic filariasis and onchocerciasis (which together affect 150 million people); whereas in veterinary medicine, the heartworm of dogs and cats is a major problem worldwide. Currently, filarial nematodes are primarily controlled by anthelminthic drugs such as ivermectin; while in many cases, diagnosis is still reliant on low-sensitivity parasitological methods. The sustainability of drug-based control programmes is threatened by contraindications, donor fatigue and the emergence of resistance. In 2007, a filaria parasitizing humans, *Brugia malayi*, was the first parasitic nematode to have its genome sequenced. However, with the advent of next-generation sequencing technologies, an explosion of filarial genomic resources has resulted recently, with 11 additional genomes now available on the WormBase ''ParaSite'', and several others at the assembly stage. We have been keen to exploit these resources for vaccine and diagnostics development, especially in the case of human onchocerciasis (river blindness) that remains a significant public health problem in sub-Saharan Africa. Using the model filaria *Litomosoides sigmodontis* in rodents and a natural parasite of African cattle, *Onchocerca ochengi*, we have characterised secretomes from several lifecycle stages and identified novel proteins with potential as immunoprophylactic or immunotherapeutic agents, as well as proteins released into host body fluids that may be suitable as diagnostic biomarkers. Furthermore, the identification of a unique gene duplication in the human parasite *Onchocerca volvulus* has enabled discrimination from *O. ochengi* in infected blackfly vectors via a loop-mediated isothermal amplification assay, which could be used for monitoring the status of control programmes. Finally, sequencing of small RNA libraries from *O. ochengi* secretions has facilitated the identification of circulating *Onchocerca* microRNAs in the blood of human onchocerciasis patients, suggesting that molecular diagnosis of onchocerciasis may be in reach. The further application of genomic resources to the grand challenge of filarial disease control will accelerate global eradication efforts.

63. DEVELOPING AUTOMATED METHODS FOR ANALYSIS AND SORTING OF *GLOBODERA PALLIDA* CYSTS, EGGS, AND JUVENILES USING THE COPAS 1000 LARGE PARTICLE FLOW CYTOMETER. **Malinouski, M.[1], J. Rowley[2], and L.M. Dandurand[2].** [1]Union Biometrica Inc. 84 October Hill Rd, Holliston, MA 01746; [2]Department of Plant, Soil and Entomological Sciences, University of Idaho 875 Perimeter Drive MS 2339, Moscow, ID 83844.

Potato cyst nematodes, such as *Globodera pallida,* are economically important plant parasites and threaten global food security. *G. pallida* is also a valuable model organism to study the biology of cyst nematodes. Handling large quantities of *G. pallida* for screening purposes is a labor intensive process. Here we report the development of an automated method for the purification of *G. pallida* cysts from soil samples using a COPAS 1000 large-particle flow cytometer. Using this method, we purified 72 cysts in approximately 5 minutes, and confirmed purity by microscopic observation. We were also able to isolate, analyze, and sort various populations of *G. pallida* eggs. Two distinct populations of eggs were identified based on red autofluorescence (AF) signal. A 15-fold difference in fluorescence intensity (ex. 488nm, em. 615nm) was noted between the two populations. Eggs exhibiting low red AF were verified as living and high red AF were verified as dead using Meldola's Blue staining. Further, the COPAS 1000 flow cytometer was used to accurately sort user-specified numbers of juvenile worms to wells of 96-well plates. We have developed a method for the high-throughput analysis and sorting of different populations of potato nematode cysts, eggs, and juvenile worms on the basis of size, optical density, and fluorescent parameters. We were able to identify and purify cysts from soil/root particles, distinguish viable and dead eggs, and sort precise numbers of juvenile worms into desired collection receptacles.

TEACHING NEMATOLOGY THROUGH AN EXPERIENTIAL APPROACH IN UNDERGRADUATE EDUCATION. **Marahatta, S.P.[1], B. Yamamoto[1], V.H. Henmi[1], C.L. Martiney[1], D.K. Foley[1], P.V. Fewkes[1], R. Peterson[1], J-H. Lau[2], and K. Johnson[3].** [1]Kauai Community College, 3-1901 Kaumualiĩ Highway, Lihue, HI 96766; [2]University of Hawaii at Manoa, Department of Plant and Environmental Protection Sciences, 3050 Maile Way, Honolulu, HI 96822; [3]Kauai High School, 3577 Lala Road, Lihue, HI 96766.

Benefits of experiential teaching in undergraduate education in agriculture have been advocated by academic institutions. However, undergraduate student focused experiential teaching, specifically in a specialized field of agriculture such as nematology is in a preliminary stage. Thus, a research-based teaching strategywas used for mentoring undergraduate students enrolled in an internship course, Plant Bioscience Internship (PBT 290V), at Kauai Community College in summer 2012-spring 2015. One of the student learning objectives (SLOs) of the course was to develop students' research and presentation skills. Students were mentored in identification and management of multiple plant-parasitic nematodes including reniform nematode (*Rotylenchulus reniformis*), root-knot nematode (*Meloidogyne* spp.) and burrowing nematode (*Radopholus similis*), and the dominant bacterivorous nematodes, Rhabditidae, through a non-chemical nematode management approach, using sunn hemp (*Crotalaria juncea*) cover cropping or vermicomposting in each semester. At the end of the course, internship results were analyzedand presented in workshops. Internship findings were published in workshop proceedings and/or peer-reviewed journals by 15.38% and 61.53% of the enrolled students (N=13), respectively. In each year, course SLOs were assessed through comparing the Kauai Community College designed Course Assessment Report of Data (CARD) records. Year-wise course SLO values were not different ($P > 0.05$). However, other indicators such as conference attendance by students and student publications could serve as better indicators for assessing a course taught in an experiential approach. Experiential teaching and learning in nematology could be successfully incorporated in undergraduate students' education.

CAN SUNN HEMP BE USED AS A MODEL PLANT FOR EXPERIMENTAL TEACHING AND LEARNING? **Martiney, C.L.[1], S.P. Marahatta[1], and K. Johnson[2].** [1]Kauai Community College, 3-1901 Kaumualiĩ Highway, Lihue, HI 96766; [2]Kauai High School, 3577 Lala Road, Lihue, HI 96766.

Academic institutions need a model plant that is useful as a teaching tool for the study of plant and nematode biology inside the laboratory as well as in the field. Sunn hemp (*Crotalaria juncea*) (SH), a tropical leguminous cover crop, is a possible option to meet both needs as it is most commonly used in the field, but can also be used as a potting soil amending material inside the shadehouse or laboratory. In the field, SH has been used as a cover crop because it releases an allelopathic compound known as monocrotaline when incorporated into the soil, which helps to suppress plant-parasitic nematodes. Additionally, SH enhances beneficial nematodes, and improves soil health. However, the allelopathic effects of SH could also reduce cash crop seed germination if SH is planted immediately after cover cropping. To find out the optimum interval of days between SH cover cropping and cash cropping, a field experiment and a shadehhouse experiment were conducted in 2013. In the field experiment, SH and pigeon pea (*Cajanus cajan*) (PP) cover crops were separately grown for 1 month, tilled and incorporated into the soil. During the cover cropping period, a separate plot with no cover crop fallow was maintained as a control (CC). Corn, *Zea mays*, were seeded immediately after incorporating cover crops into the soil. In the shadehhouse experiment, fresh SH was incorporated with soil samples at 1.0% (w/w) (SH+) or non-incorporated (SH-), placed in a 10-cm-d plastic pot, and left for 0 (W-0), 1 (W-1) and 2 (W-2) weeks. At W-0, W-1 and W-2, corn seeds were planted. In the field experiment, compared to PP and CC, SH reduced corn germination ($P < 0.05$). In the shadehouse experiment, compared to SH(-), SH(+) reduced corn germination at W-0 ($P < 0.05$), but not at W-1 and W-2 ($P > 0.05$). When farmers use SH as a cover crop in the field, it is best to wait 1 week before seeding a cash crop in order to optimize seed germination. In addition to this, because of the known effects of SH on beneficial nematodes, an outreach was conducted with high school students to determine if SH would be an effective tool for teaching plant and nematode biology. With the involvement of high school students, two experiments were conducted using dried SH foliage powder incorporated with soils sampled from the school campus at 1.0% (w/w) (SH+) or non-incorporated (SH-), placed in a 10-cm-d plastic pot, and held for 1 week, before being transferred to a Baermann funnel for an additional week. The beneficial nematodes, Rhabditidae, were then counted using an inverted microscope with both (SH+) and (SH-) samples and compared. SH(+) consistently enhanced Rhabditidae number ($P > 0.001$). Instructors could use SH as a teaching tool for plant and nematology related courses in colleges and high schools.

THE *HETERODERA GLYCINES* CYST AS A SOURCE OF NATURAL PRODUCTS: PROTEASES AND THEIR INHIBITION. **Masler, E.P. and D.J. Chitwood.** Nematology Laboratory, USDA, ARS, NEA, Beltsville MD 20705.

We are interested in development and hatching in cyst and root-knot nematodes, and the effects of the environment on the mechanisms involved. Understanding nematode-environment interactions at the biochemical and molecular levels is essential for novel control agent discovery. Female cyst nematodes offer extraordinary opportunities to examine these interactions, since they exist at the convergence of host, nematode internal, and rhizosphere environments. Cysts and their association with embryogenesis, dormancy and hatching present particularly rich targets for exploration. *Heterodera glycines* cysts contain proteases, and protease inhibitors that affect cyst and juvenile proteases differently (Masler 2015, *Nematology* 17: 91-102). Here we present results from an expanded analysis of the proteases of the *H. glycines* cyst. Cyst content was

prepared by gently extracting crushed cysts in deionized and distilled water, centrifuging the extract (40000 x g), and vacuum drying the 40000 x g supernatant. This native cyst extract (CE) preparation (HglnCE) was used in enzyme screens. Heated preparations (HglhCE) were used as inhibitor sources. HglnCE was screened against a FRET-peptide library (REPLi, Mimotopes, Australia) designed for drug discovery and based upon cleavage site sequences for 4 endoprotease types. The library consisted of 3400 unique sequences assembled into 512 sample pools of related sequences. Each pool was qualified by the manufacturer to contain substrates for 1 or more protease types: aspartic, cysteine, metallo, serine. We used data provided from the manufacturer to map all 512 samples with regard to type of protease targeted, and used this map to profile HglnCE protease content. The HglnCE enzymes digested peptides in 96% of the sample pools, representing all 4 protease types, and with a broad range of relative activities. Vmax/min/ug varied > 60-fold between lowest and highest digestion rates. To simplify analysis, we looked at those samples (n = 181) where response was > Vmax mean. Among these samples, HglnCE digestion was indicated for cysteine (n = 53), serine (n = 98), metallo (n = 123), aspartic (n = 42), and unidentified (n = 15) proteases. The sum is greater than 181 because of multiple protease types indicated in some sample pools. Limiting analysis to those samples that were digested at > 2x Vmax mean (n = 40), and adjusting for multiple activities, we found that HglnCE endoproteases are distributed as follows: metalloproteases (37%), serine proteases (32%), aspartic proteases (18%), cysteine proteases (12%), and unidentified (1%). Using this information, we have begun an examination of specific proteases and their inhibition, beginning with metalloproteases. Early results indicate that HglhCE inhibits matrix metalloprotease activity in HglnCE by 13%, whereas inhibition is 40% in *H. glycines* J2 extracts and as high as 80% in *Meloidogyne incognita* J2 extracts. Identification of HglhCE inhibitors will be important in evaluating them as potential new nematode control agents. Characterization of these inhibitors and HglnCE proteases, and their possible interactions on developing embryos, will further elucidate the specific roles of the cyst in nematode development. These and other benefits of cyst components exploration are discussed.

EFFECT OF SOME FUNGI AND PLANT GROWTH PROMOTING RHIZOBACTERIA (PGPR) ON PLANT GROWTH AND MELOIDOGYNE INCOGNITA REPRODUCTION IN ISMAILIA, EGYPT. **Massoud, S.[1], S.H. Hassan[2], T.S. Abdelmonem[1,3], A.E. Khalil[2], and H.M. Abdelnabbi[1].** [1]Agricultural Botany Dept., Faculty of Agriculture, Suez Canal University, Ismailia, Egypt; [2]Plant Nematology Research Dept., Plant Pathology Institute, Agriculture Research Center (ARC), Egypt; [3]Biological Science Dept., Faculty of Science, University of Jeddah, Saudi Arabia.

Four fungal species (*Arthrobotrys* sp., *Hirsutella rhossiliensis, Trichoderma harzianum, T.viride)* and six bacterial isolates (*Paenibacillus polymyxa, Bacillus thuringiensis, B. megaterium, B. subtilis, Serratia* sp., Pseudomonas fluorescens) were selected to determine their ability to suppress the reproduction of *Meloidogyne incognita* on susceptible grapevine plants [*Vitis venifera* L. (cv. Flame Seedless)] under greenhouse conditions. The susceptible grapevine plants were treated by all previous microorganisms individually by rate 1×10^8 CFU/20ml. The bio-agents inoculums were added to plants by two different ways. The first depends on adding the bio-agents only once (one week before nematode inoculation; $3000J_2$/plant) and the second adding the bio-agents twice (one week before and one week after nematode inoculation). Nematicide treatment, VYDATE® as liquid 0.5% was used for comparison. All tested treatments (one week before nematode inoculation) significantly decreased number of J_2 in the soil by rate 83%, number of root galls and number of egg-masses per plant 83.7, 85% respectively. The highest reduction was evident with *Bacillus subtilis,* in reducing root galling and number of egg-masses per plant. *B. thuringiensis*, *Paenibacillus polymyxa* and the fungus *Arthrobotrys* sp., gave the same effect in reducing root galling and number of egg-masses per plant. The least effective bio-agents in reducing nematode population were recorded in *Trichoderma harzianum* and *B. megaterium*. On the other hand when the fungi and bacteria added twice (one week before and one week after nematode inoculation), that increased the effect of bio-agents in reducing root galling and number of egg-masses per plant. *B. megaterium* was followed by the fungus *Arthrobotrys* sp., since they gave better results more than nematicide treatment (VYDATE® 0.5%). The number of second stage juveniles in the soil was differ according to the different treatments. Fresh weights of root, shoot and their length were taken as a criteria for grapevine growth response. Plant treated by *B. thuringiensis*, one week before nematode inoculation gave the highest increase in the whole plant weight followed by *B. subtilis*. On the other hand when the bio agent were added twice, the highest increase in the whole plant was obtained in plant treated by *Arthrobotrys* sp., and by *Hirsutella rhossiliensis*, followed by *B. thuringiensis*.

SOIL HEALTH DIFFERENCES IN THREE AFRICAN SOIL GROUPS REVEALED BY NEMATODE COMMUNITY ANALYSIS. **Maung, Z.T.Z.[1], S. Yildiz[1], W. Kimenju[2], C. Kwoseh[3], V. Saka[4], and H. Melakeberhan[1].** [1]Agricultural Nematology Laboratory, Department of Horticulture, Michigan State University, East Lansing, MI 48824, USA; [2]University of Nairobi, P.O Box 30197, G.P.O, Nairobi, Kenya; [3]Kwame Nkrumah University of Science and Technology, PMB, UPO, Kumasi, Ghana; [4]Lilongwe University of Agriculture and Natural Resources, Lilongwe, P.O. Box 219, Malawi.

A soil group (order) based understanding of biological degradations could lead to developing scalable remedial and/or preventive soil health management strategies to deal with the vast degradations in sub-Saharan Africa (SSA). While the same soil groups may have overlapping textures (% sand, silt and clay), they have different horizons that correlate well with ecological zones. Using nematode and microbial communities as indicators of soil health, we analyzed soil samples from

undisturbed (pristine forest or natural vegetation) and disturbed (agricultural or grazing) landscapes in Ferralsol, Lithosol and Nitosol soil groups in Ghana, Kenya and Malawi. A total of 74, 69, and 77 nematode genera were detected in Ghana, Kenya and Malawi, respectively. Only 12%, 20 – 25%, 25% and 56% of omnivore, fungivore, herbivore and bacteriovore trophic groups, respectively, were common to all three countries. The herbivores *Amplimerlinius, Heterodera* and *Trophurus* in Ghana, *Paratrophurus* in Kenya, and *Trichodorus* and *Longidorus* in all three countries were present in Ferralsols only. Total abundance across soil groups and countries ranged from 38 to 248 nematodes/100 cc of soil, with the most numbers observed in Nitosols. Generally, nematode diversity was higher in natural than in disturbed landscapes, suggesting impact of land use practices on biological diversity and degradations in the soil groups. Maturity indices indicated that the Malawi and Kenya soils were more disturbed than Ghana, and Ferralsols appeared to be more disturbed than Lithosols. The fertility index across soil groups in Ghana was closer to natural (0.9) than in Kenya and Malawi, which reflects the range of time that the soils have been in use. Soil food web structure showed similar level of depletions across landscapes, soil groups and countries, suggesting that these soil groups are naturally fragile to meet agroecosystem expectations. There were varying levels of two- and three-way interaction effects on the biological parameters, suggesting the conditions vary by landscapes, region and/or soil groups. Furthermore, multi-factor correlation analyses of nematode abundance and frequency, soil texture and physiochemical properties showed distinct separation of the soil groups and by country, and Ferralsols further from Lithosols and Nitosols. Although biologically degraded, the results indicate that these soil groups have different biological properties and may not respond the same way to a given treatment. The study for the first time establishes a biological basis for designing soil amendment strategies that could potentially lead to improving soil health conditions across soil groups and climatic zones.

EFFECTS OF ROTATION AND COVER CROPS ON NEMATODE COMMUNITIES AND SOIL HEALTH IN DIFFERENT SUGAR BEET PRODUCTION SOILS. **Maung, Z.T.Z.[1], S. Poindexter[2], G. Clark[3], J. Stewart[3], L. Hubbell[3], and H. Melakeberhan[1].** [1]Agricultural Nematology Laboratory, Department of Horticulture, Michigan State University (MSU), East Lansing, MI 48824, USA; [2]Extension Agriculture and Agribusiness, Michigan State University (MSUE), Saginaw, MI 48607, USA; [3]Michigan Sugar Company (MSC), Agricultural Research Center, Bay City, MI 48706, USA.

Managing sugarbeet cyst nematode (SBCN, *Heterodera schatii*) and improving soil health (organic matter, biological, physiochemical, nutritional, and water holding priorities) are priorities for the Great Lakes Region sugarbeet industry. Crop rotation and use of cover and trap cropping systems are among the practices that potentially address both priorities. The pressing priorities and the practices that might lead to solutions, however, are further complicated by the diversity of sugarbeet production soils, cropping systems and varying infestations of SBCN. For example, it is unknown how any of cover, trap or rotation crops relate to the two industry priorities in different soils. The objective of this study was to compare how rotation (corn and soybean) and cover and trap (oil seed radish and mustard) crops affect soil health, nematode community and sugarbeet production in sandy clay loam and loam soils. A study was conducted in 2013 and 2014 using a randomized complete block (RCB) design of nine crops (oilseed radish: Defender and Tillage; mustard: Pacific Gold and Ida Gold; soybean: 92Y80 and 92M91; sugar beet: SBCN-tolerant, B-18RR4N and –susceptible, B-10RR34; and corn: P9910R) and six replicates. Nematode community composition, soil physiochemical properties, sugar beet yield (% of sugar, % of sugar purity, and recoverable white sugar per ton), and biomass of all crops were measured. Recoverable white sugar per ton and percentage of sugar from both sugar beet varieties on loam soil in 2013 were the lower than in 2014. Percentage of sugar purity of both varieties in 2014 on sandy clay loam soil was significantly lower than in the loam soil. Except for susceptible soybean on loam soil, populations of cyst nematode (*Heterodera* spp.) in the soil was less in 2014 than in 2013 for all crops on both soils. However, there was no significant difference among crops in the same growing season within the same soil. Based on the composition of nematode assemblages, soil food web indices: Enrichment (EI), Structure (SI), Basal (BI), and Channel (CI) indices, were calculated to infer soil food web condition. Fertility index varied among crops. While not statistically significant, percent composition of nematode trophic groups showed some variation across growing season and soil type. The soil food web analysis showed that both soil types do not have ideal conditions for agroecosystem suitability (few data points in Quadrant B). However, nematode communities in the sandy clay loam were more stressed than the loam soil and the stress varied by crop as well. Principal component analysis showed a distinct pattern of correlation between crops grown in different soil types with nematode community indices and soil physiochemical properties. The study suggests that more emphasis should be given to location-specific factors.

INCREASE IN THE INCIDENCE OF SYMPTOMS OF PINE WILT DISEASE IN SOUTHEAST LOUISIANA. **McGawley, E.C.[1], C. Overstreet[1], and Y. Takeuchi[2].** [1]Department of Plant Pathology and Crop Physiology, Agricultural Center, Louisiana State University, Baton Rouge, LA 70803, USA; [2]Laboratory of Terrestrial Microbial Ecology, Graduate School of Agriculture, Kyoto University, Kyoto 606-8502, Japan.

The nematode *Bursaphelenchus xylophilus* was first described as *Aphelenchoides xylophilus* by Steiner and Buhrer in 1934 from fungal cultures derived from logs of *Pinus palustris* in Bogalusa, Louisiana. Since 1979 and the first report of the nematode from Austrian and Scotch pine in the state of Missouri, nematologists in America have monitored the nematode

closely in spite of the fact that it is probably an indigenous species. Along Interstate 12 in Louisiana, which is an intrastate highway that spans about 86 miles in an east-west direction, foliage of pine trees frequently displays the yellow-brown symptoms associated with Pine Wilt Disease (PWD) and other biotic and abiotic diseases/disorders. Over the last five years, such symptoms have become more common and sampling has indicated an incidence of the nematode and pine sawyer beetles in 14 percent of samples. In the Baton Rouge metropolitan area, where the primary *Pinus* species are *P. taeda* and *P. elliotti*, the occurrence of foliar symptoms of PWD and isolation of the nematode are significantly greater. The nematode has been isolated from 36% and of samples collected to date and longhorn beetles (*Monochamus carolinensis*) have been collected from 8% of samples from branches of symptomatic trees. Individuals of *M. carolinensis* macerated and placed on Baermann funnels rarely contain *B. xylophilus*.

EFFECT OF VERMICOMPOST TEA ON PLANT-PARASITIC AND BENEFICIAL NEMATODES. **Mishra, S., B.S. Sipes, and K-H. Wang.** Department of Plant and Environmental Protection Sciences, University of Hawaii at Manoa, Honolulu, HI.

Although compost tea prepared from cured or aged vermicompost contains more mineralized nutrients that are more available for plant to uptake, a preliminary study showed that compost tea from uncured vermicompost is better in suppressing plant-parasitic nematodes. A study comparing vermicompost tea (VCT) from uncured, 1-, and 6-month cured vermicompost to untreated control showed that VCT from uncured and 1-month cured vermicompost suppressed root-knot nematodes (RKN), *Meloidogyne incognita*, infection better than VCT from 6-month cured vermicompost and the control. However, effects of drenching vermicompost tea on suppressing plant-parasitic nematodes are inconsistent, varying from no effect, short-term effect, to significant effect. One working hypothesis is that drenching VCT prepared from uncured VC temporarily induced host plant resistance. A greenhouse split-root experiment was conducted to examine the induction of host-plant resistance against RKN infection on cucumber (*Cucumis sativus*). One side of the split root was either drenched with VCT or water, and the other side of the roots was inoculated with 200 second-stage juveniles of RKN. Experiment was arranged in completely randomized design with 5 replications. Nematode penetration was significantly lower on vermicompost tea drenched plants ($P < 0.05$) than the water treatment. However, another preliminary study indicated that this induction of host plant resistance by VCT did not last for more than a month. A 2 × 4 (cover crop × VCT drenching frequency) split-plot experiment was conducted in a root-knot nematode infested field. The cover crop (CC), tiller radish (*Raphanus sativus*), grown for 1 month was terminated prior to cucumber planting. Cucumber seedlings were drenched with VCT prepared from uncured vermicompost at 1, 2, or 4 weeks interval and compared to drenching with water. Experiment was arranged in randomized complete block design with 4 replications. Nematode abundances were monitored on each plot at 2-month interval after cucumber planting. Drenching VCT at weekly interval suppressed RKN ($P < 0.05$) but its effects against reniform nematodes (*Rotylenchulus reniformis)* was not significant. Drenching VCT at 2-week interval enhanced abundance of *Ecumenicus*, an omnivorous nematode, in cover crop but not in no cover crop plots. However, drenching vermicompost tea weekly increased nematode richness only in no cover crop plots. These results supported our hypothesis that drenching VCT prepared from uncured vermicompost can induce host plant resistance against RKN if drenched weekly, but this VCT did not improve cucumber yield. Vermicompost tea did improve soil food web structure as indicated by higher abundance of omnivorous nematodes. Thus, future experiment needs to be followed up at the same experimental site to examine a longer-term effect of drenching VCT from uncured vermicompost on crop yield.

EFFICACY OF FLUENSULFONE IN A TOMATO-CUCUMBER DOUBLE CROPPING SYSTEM. **Morris, K.[1], D. Langston[1], D. Dickson[2], R. Davis[3], P. Timper[3], and J. Noe[4].** [1]Department of Plant Pathology, University of Georgia, Tifton, Georgia, USA; [2]Department of Entomology and Nematology, University of Florida, Gainesville, Florida, USA; [3]USDA ARS, Crop Protection and Management Research Unit, Tifton, Georgia, USA; [4]Department of Plant Pathology, University of Georgia, Athens, Georgia, USA.

Field trials were conducted in the spring and fall of 2013 and 2014 in Tifton, GA and Citra, FL to evaluate the efficacy of different control methods for nematodes in tomato-cucumber double cropping systems. The purpose of these trials was to determine what effect nematode control strategies applied to tomato in the spring have on nematode population levels and damage on a second crop of cucumber in the fall. Treatments in the spring were 1,3 dichloropropene (1,3-D) (112.0 L/ha), fluensulfone (3.0 kg a.i./ha), a resistant cultivar ('PS 01522935'), and an untreated control. All plots except for those planted to the resistant cultivar were planted with the cultivar 'Tribute'. The number of J2s in the soil, plant vigor, yield, incidence, and galling severity ratings (0-10 scale) were recorded for spring and fall crops. There was no location x treatment interaction for tomato vigor, weight, galling or incidence data between locations so data was combined each year ($P>0.05$). There was no effect of nematicide treatment on vigor or yield of tomato. The 1,3-D, fluensulfone, and the resistant cultivar significantly decreased root galling by 91%, 73%, and 97%, respectively compared to an untreated control. The 1,3-D, fluensulfone, and resistant cultivar reduced galling incidence by 77%, 41%, and 89%, respectively compared to an untreated control. Tomato plots from the spring were divided into split-plots for the fall where the main plot was the treatment from the spring cucumber subplots were either treated with fluensulfone (3.0 kg a.i./ha. via drip irrigation) or left untreated. The fall application of

fluensulfone significantly improved cucumber vigor and significantly reduced gall ratings compared to untreated subplots. The fall treatment increased cucumber yield in Citra, but not in Tifton. The results suggest that fluensulfone can be a valuable tool for management of root-knot nematode in double cropping systems.

INCREASE IN THE INCIDENCE OF SYMPTOMS OF PINE WILT DISEASE IN IDENTIFICATION AND CHARACTERIZATION OF TWO NOVEL EFFECTORS FROM ROOT-KNOT NEMATODES. **Muthreich, M., N. Leelarasamee, J. Utermark, and C.A. Gleason.** Georg-August-University, Göttingen, Schwann-Schleiden-Forschungszentrum, D-37077 Göttingen, Germany.

Some of the most economically damaging plant-parasitic nematodes belong to the group of root-knot nematodes (*Meloidogyne* spp). These nematodes have an extremely broad host range and can be serious pests on major food crops. In order to establish a successful infection, the nematode secretes a large repertoire of effectors that suppresses plant defences and/or alters host cell physiology. To identify the effector complement of the root-knot nematode, we performed bioinformatic analyses of the known root-knot nematode secretome and genome(s). We identified several novel genes from the nematode that, when heterologously expressed by a bacterial pathogen, *Pseudomonas syringae pv. tomato* DC3000 (*Pst DC3000*), can enhance bacterial virulence in Arabidopsis. This suggests that certain nematode proteins are secreted into the plant and targeting conserved plant immune responses. Therefore, when these nematode genes are expressed in bacteria, they contribute to the bacterial pathogenicity and virulence *in planta*. From our initial screen of nearly 20 nematode genes in the heterologous bacterial system, we have focused our attention on two root-knot nematode genes, Mh265 and Mi131. Mh265 is a gene specific to root-knot nematodes that encodes a novel secreted nematode protein. Mi131 is found in several species of nematodes and contains a conserved, actin-binding domain. Interestingly, we found that ectopic expression of these putative effectors in Arabidopsis enhanced plant susceptibility to root-knot nematodes, suggesting that they both play important roles in nematode pathogenicity in plants. To identify the plant targets of our effectors, we used a yeast two hybrid assay to screen plant cDNA libraries with either Mh265 or Mi131 as bait. We discovered that Mh265 interacts with a plant protein involved in vesicular transport, highlighting the possibility that the nematode may be manipulating intercellular trafficking, a process linked to plant immunity. The other effector of interest, Mi131, could specifically bind to plant actin, suggesting that the nematode is secreting effector(s) that can directly affect the plant cytoskeleton. Thus, by using a bacteria-based screen, we have identified two new nematode effectors, providing important information to further our understanding of the complex interaction between plants and root-knot nematodes.

SCREENING EDIBLE GINGER AND TURMERIC CULTIVARS FOR RESISTANCE TO ROOT-KNOT NEMATODES. **Myers, R., C. Mello, and L. Keith.** USDA ARS DKI-PBARC, 64 Nowelo St., Hilo, HI 96720.

Twenty-two edible ginger and turmeric cultivars were screened for resistance or tolerance to *Meloidogyne incognita*. Plants were raised in 66 L grow bags in greenhouses in Hawaii according to established practices for producing bacterial wilt-free ginger. Three months after planting, each grow bag was inoculated with 2,000 *M. incognita* eggs. The trial was conducted in 2013 and repeated in 2014. In the first year, yields were reduced by an average of 50% among inoculated plants as compared to uninoculated controls. The highest susceptibility was observed in ''Black turmeric'' and ''True white'' ginger with yield losses of 80%. Edible ginger cultivar ''I'' from the PBARC germplasm collection demonstrated the highest tolerance to the nematodes. ''Black turmeric'' had the highest number of J2s in the soil and differed from all cultivars except ''Blue finger''. Final nematode populations did not differ among the other cultivars. The greatest number of root-knot nematode eggs was recovered from turmeric cultivar ''BDT'' followed by ''Blue finger''. ''BDT'' produced the most eggs per gram of root whereas ''Blue finger'' had the greatest root weight. Few differences in response to *M. incognita* were observed among the edible ginger germplasm. No resistance to *M. incognita* was discovered in any of the ginger or turmeric cultivars tested.

BROAD-BASED ROOT-KNOT NEMATODE RESISTANCE IN THE SOUTHEASTERN AFRICA COWPEA GENE POOL. **Ndeve, A.[1], W.C. Matthews[1], J.R.P. Santos[1], R.M. Chiulele[2], T.J. Close[3], and P.A. Roberts[1].** [1]University of California Riverside, Dept. Nematology, Riverside CA 92521, USA; [2]Eduardo Mondlane University, Dept. Crop Production, Maputo 257, Mozambique; [3]University of California Riverside, Dept. Botany and Plant Sciences, Riverside CA 92521.

Cowpea (*Vigna unguiculata* L. Walp) is an inexpensive source of proteins and important for food security in Africa. However, infection by root-knot nematodes (RKN), *Meloidogyne incognita* and *M. javanica* suppress cowpea yield, and selection for virulence limits effectiveness of available *Rk* resistance genes. Fifty-three southeastern African genotypes (land-races and accessions), comprising cowpea gene pool 2, were screened for resistance to RKN in field, greenhouse and growth pouch tests. Response of cowpea genotypes to root galling (GI) and egg mass production (EM) showed that seven genotypes were consistently highly resistant (ANOVA, $P < 0.05$) to both RKN species. Virulence indexes, based on GI and EM production on the resistant genotypes as a proportion of GI and EM on a susceptible genotype, ranged from 6.24-30.8 % and 1.25-15.81 %, respectively, with *M. javanica* and 0-2.40 % and 0.20-1.85 %, respectively, with *Rk*-avirulent *M. incognita*. Virulence index, based on GI, of an *Rk*-virulent *M. incognita* on resistant cowpeas ranged from 11.07 to 39.48 %. GI and EM of the avirulent *M. incognita* on resistant cowpeas did not differ from that on control plants with gene *Rk*. The novel resistant genotypes had lower ($P < 0.05$) GI and EM

induced by *M. javanica* than the control plants with gene *Rk*. Differences in GI and EM between the resistant genotypes and breeding lines carrying stacked *Rk*-genes were not significant ($P > 0.05$). The identified resistant genotypes are sources of effective RKN resistance for cowpea breeding; however, their uniqueness from known sources is still to be determined.

NEMATODES AS INDICATORS OF ECOSYSTEM RESPONSES TO ALTERED PRECIPITATION REGIMES: OBSERVED RESPONSES AND FUTURE PERSPECTIVES. **Nielsen, U.N.** Hawkesbury Institute for the Environment, University of Western Sydney, Penrith NSW 2751, Australia.

Global changes are considered the greatest threat to Earth's ecosystems and changes in land use, temperature and precipitation have already been observed to impact ecosystems. Our ability to quantify the impacts of such changes is, however, relatively limited, particularly when ecosystem responses are small. This is problematic, as even small changes may signal more dramatic long-term impacts warranting the adaptation of better indicators of ecosystem responses to global changes. Nematodes are ubiquitous in terrestrial ecosystems, generally occur in great abundances, represent a very broad range of functional types, and are well established as being highly important to ecosystem function. Nematodes can be used as indicators of ecosystem state and several indices such as the maturity index and the plant parasite index have been developed to provide insights into the functioning of ecosystems in response to land use and other perceived disturbances. I will discuss the use of nematodes as indicators of ecosystem responses to altered precipitation regime, building on a review of current literature and the results of grassland rainfall manipulation experiment (DRI-Grass) at University of Western Sydney, Australia with samples collected in April, 2015. Nematodes ought to be useful indicators of ecosystem responses to altered rainfall regime and the fact that several studies have shown nematode community composition to be strongly related to precipitation supports this hypothesis. However, recent work shows idiosyncratic responses to artificial changes in precipitation at the trophic level, and often no responses are observed. Similarly, two years of imposed changes in precipitation (increased and decreased precipitation, altered frequency, seasonal drought) in the DRI-Grass experimental framework had limited impacts on total or trophic level nematode abundances despite great treatment effects on the vegetation aboveground and edaphic variables. The only observed change was a slight, albeit significant, increase in the relative abundance of bacterial feeders in response to seasonal droughts indicating that this treatment caused a change in the decomposition pathway. Hence, nematode trophic level abundances appear to be highly resistant to altered precipitation regimes and does not provide great insight into effects on ecosystem functioning. Further ongoing analyses of the nematode community responses at the species/genus level will provide more insight into the use of nematodes as indicators of altered precipitation regime. It therefore appears that the use of nematodes as indicators will require knowledge of life history traits at relatively high taxonomic resolution (i.e. species or genus level).

FLUENSULFONE FOR MANAGEMENT OF *MESOCRICONEMA XENOPLAX* ON PEACH. **Noe, J., G.B. Jagdale, W.T. Holladay, and P.M. Brannen.** Department of Plant Pathology, University of Georgia, Athens, GA, USA 30602.

Mesocriconema xenoplax (Mx) is a primary causal factor of Peach Tree Short Life disease (PTSL). Pre-plant soil fumigation with 1, 3-dichloropropene is commonly used to manage PTSL by reducing soil population densities of Mx. Eventually, Mx population densities resurge to damaging levels. Alternative control methods are needed, especially nematicidal products that can be applied post-plant to extend the productive life of peach. Fluensulfone is a new nematicidal product that may have potential for post-plant application. Efficacy of fluensulfone was evaluated in two greenhouse studies. Guardian or Nemaguard peach seedlings were planted in 20 cm-dia-pots containing a greenhouse soil mix and inoculated with 2,000 Mx. Fluensulfone at a rate of 1.96 kg/ha or 3.92 kg/ha was drench applied to the soil with 300 ml water 14 days after planting (DAP) and nontreated controls (NTC) were also drenched with 300 ml water. An additional treatment of two applications of fluensulfone at 1.96 kg/ha, with the second application administered 70 days after the first application (DAA), was included in the Nemaguard trials only. Population densities of Mx were determined 30 and 70 DAA on Guardian and 30, 70, and 100 DAA on Nemaguard. Shoot and root dry weights were determined on the last sampling date. Each rootstock experiment was repeated once with 8 replications in each trial. Significant interactions were observed between treatment and experimental trial on both rootstocks, so results were not combined. Significant differences are reported at $P<0.05$. Fluensulfone application at either rate lowered Mx population densities at 30 DAA compared to NTC in both trials on Guardian. Differences were observed at 90 DAA only in the second trial, with both rates resulting in lower Mx densities. Both shoot and root weights of Guardian were lower after treatment with fluensulfone at 3.92 kg/ha compared to NTC in the second trial only. For the first experimental trial on Nemaguard, fluensulfone application at either rate lowered Mx population densities at all sampling dates compared to NTC, and two applications at the lower rate were more effective than one application at the lower rate at 100 DAA. In the second trial on Nemaguard, no differences in Mx population densities were observed at 30 DAA, but both rates of fluensulfone resulted in lower Mx densities at 70 DAA compared to NTC. In the final assay, at 100 DAA, only the treatment receiving a second application of fluensulfone at 70 DAA had lower Mx densities than NTC. In the second trial only, shoot weight of Nemaguard was higher after treatment with fluensulfone at 3.92 kg/ha compared to NTC, but root weight was highest with two treatments at 1.96 kg/ha. Fluensulfone demonstrated potential as a post-plant nematicide on both Guardian and Nemaguard rootstocks. More studies are needed to determine the effects of this product on the productive life span of peach.

NEW INSIGHTS RELATING THE SPATIAL DISTRIBUTION AND MANAGEMENT OF NEMATODES IN FLORIDA SOILS. **Noling, J.** University of Florida, IFAS, Citrus Research & Education Center, Lake Alfred, FL 33850.

Plant parasitic nematodes are very important pests of many fruit and vegetable crops in Florida. Sting and root-knot nematode are two of the most economically important nematode pests of fruit and vegetable crops grown in Florida. A variety of nonchemical and chemical strategies, particularly soil fumigation, are extensively used within an integrated system to manage these pests. Field surveys have demonstrated that upwards of 40% of strawberry acreage is infested, and of these fields, a compacted zone (traffic pan) is observed to occur just below the base of the raised, plastic mulch covered bed. In practical terms, the compacted traffic pan occurs just below the depth of the deepest tillage implement used in the field and has been shown to unavoidably cause changes in soil hydraulic conductivity, diffusion of fumigant gases, and thus soil fumigation efficacy and field distribution of nematodes and crop damage. To study the impact of subsurface compacted traffic pans, new deep core soil sampling and deep placement fumigant application technologies were developed to study the spatial distribution and management of the Sting nematode, *Belonolaimus longicaudatus*. During spring 2014, the *Probinator* was introduced to serve as a deep coring, soil sampling system capable of removing a 4 inch diameter by 40 inch deep soil core using a specialized probe and hydraulic ram system tractor mounted as a 3 point attachment. The Probinator has allowed us to study, the depth distribution of nematodes, spatial movement of soil fumigants, and causes of fumigant treatment inconsistency. In general, the nematode assay results from soil census sampling at a variety of different field sites have shown that highest sting nematode population densities are frequently observed immediately below the traffic pan at the 13 to 24 inch soil depth, but are also frequently detected at significant levels at depths of 36 inches from the soil surface. Soil population density and depth distribution of root-knot nematode *Meloidogyne hapla* was observed to be similarly distributed. Incremental analysis of soil cores with soil depth was critical to characterizing the spatial dimensions of fumigant gas movement. Only very limited fumigant movement in the water phase or as gas phase diffusion has been observed through the highly impermeable traffic pan. It would appear that current methods of fumigant treatment are very effective in reducing nematode populations in surface soil horizons. However, it would also appear that both Sting and *M. hapla* migrate to deeper soil in advance of and during the hot summer months. An apparent thermal escape mechanism contributing to nematodes survival in more ways that just heat avoidance. Our results would suggest that sting nematode damage potential may occur from migrating individuals from soil depths that are rarely studied. The potential importance of this nematode reservoir and their effects on subsequent plant growth is now being considered within the testing phases of new deep shank and subsurface drip application technologies for soil fumigants. These new systems are expected to improve fumigant penetration, overall nematode control and crop yield response consistency.

ASSESSING STING NEMATODE IMPACT AND SOIL FUMIGANT PERFORMANCE USING MEASURES OF STRAWBERRY CANOPY GREENNESS. **Noling, J.W.[1], A.W. Schumann[1], W.T. Crow[2], and M. Cody[1].** [1]University of Florida IFAS CREC, 700 Experiment Station Rd, Lake Alfred, FL 33850; [2]Dept. of Entomology & Nematology, University of Florida, Gainesville, FL 32611.

In Florida strawberry, yield losses and patchy field distributions of plant stunting are well correlated with soil population densities of the Sting nematode, (*Belonolaimus longicaudatus*). For these studies, digital imaging and multispectral reflectance were used to characterize plant stunting and strawberry yield losses to within row, green vegetative cover. A tractor mounted GreenSeeker® optical sensor was used to scan strawberry rows to provide estimates of green canopy cover (NDVI) against a backdrop of black plastic mulch covering the raised bed. Close-up, geo-referenced, digital photographs of the strawberry bed were also automatically and systematically collected at preset separations along the plant row from a boom mounted USB camera. Digital images were also collected from a compact digital camera operating in 30 Hz video mode. Camera image greenness and Greenseeker NDVI were compared using regression analysis with and without regard to digital image quality. Even though image quality was quite poor, percent vegetative cover computed from green pixels was still very descriptive of NDVI, explaining 75% or more of the variation between the two parameters. Regression analysis using images which minimize boom or interior shading, washout, or images which include large portions of the row middle (off center) removed much of the variation and improved descriptive capability to 91%. Accurate maps and assessments of fumigant treatment performance, GPS location, and sting nematode stunting severity of strawberry plants was well described by digital imaging and NDVI field mapping of experimental fields. These results illustrate how digital imaging and greenness analysis can be used in lieu of NDVI to provide a quantitative measure of strawberry yield and to provide growers guidance on suitable alternatives to methyl bromide soil fumigation for nematode management. We are also confident that the USB cameras will add value to automated field surveys of differences in strawberry canopy size, disease and insect feeding damage, and may eventually serve as real-time sensors for on-the-go smart spraying in Florida strawberry.

BEHAVIORS TO FATTY ACID DRIVED FROM THE HOST BUG IN *CAENORHABDITIS JAPONICA*. **Okumura, E.[1,2], R. Ozawa[3], and Y. Takeuchi[2].** [1]Institute for Integrated Cell-Material Sciences, Kyoto University, Katsura Int'tech Center, Kyoto daigaku-katsura, Nishikyo, Kyoto 615-8530, Japan; [2]Graduate School of Agriculture, Kyoto University, Kitashirakawa Oiwake, Sakyo, Kyoto 606-8502, Japan; [3]Center for Ecological Research, Kyoto University, Hirano 2-509-3, Otsu, Shiga 520-2113, Japan.

A bacteriovore nematode, *Caenorhabditis japonica* forms a phoretic and necromenic association with the subsocial burrower bug, *Parastrachia japonensis* in a species-specific manner. *C. japonica* dauer larvae (DL) attach to nymphs of *P. japonensis* around the nest under leaf litter, and stay on the body surface as quiescent stage for long term. In the previous study, we found that *C. japonica* DL were attracted to hexane extracts of the body surface components of *P. japonensis* and 4 chemical components which were detected by GC-MS as species-specific candidates. In this study we compared the hexane extracts of mother and nymph bug to determine if they differed in function as a cue for DL behavior. Hexane extracts prepared by washing *P. japonensis*'s body surface with hexane were analyzed by GC-MS. Mother and nymphs were sampled when mother hold her egg mass. After nymphs hatched, the mother and approximately 20 individuals of 2nd or 3rd stage of nymphs per each sample were used for an extraction. In total 5 pairs were analyzed. By calculating the mean ± SE of the data, we found that 4 fatty acids were especially detected in samples from nymphs. Bioassay was done on a 6-cm NGM plate with 1-cm diam. test and control spots near the edge the plate. After 6 μl of test and control were supplied, *C. japonica* DL were placed onto the center of the plate. We counted the number of nematodes on test and control spots every 10, 30, and 60 min. The attractiveness was evaluated as Chemoattraction Index value [(the number of nematode on test – the number of nematode on control) / total number of nematodes]. In addition, we examined the effects of the fractions on arresting dauer larvae dispersal by inoculating *C. japonica* DL on the center of a 6-cm NGM plate where the sample was spotted in advance. In our presentation, we will discuss the effectiveness of 4 fatty acid derived from host nymphs to *C. japonica* DL, and the possibility whether DL choose their host stage consciously or not.

PASTEURIA SP. ENDOSPORE ATTACHMENT TO *PRATYLENCHUS* SPECIES. **Oliveira, C.J.[1], Z. Grabau[2,3], D.A. Samac[2], and S.Y. Chen[2,3].** [1]Nematology Laboratory, Federal University of Goiás, Goiânia, Brazil; [2]Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108; [3]Southern Research and Outreach Center, University of Minnesota, Waseca, MN 56093, USA.

Pasteuria spp. are endospore-forming bacteria, and most of them are obligate parasites of nematodes. A number of studies have demonstrated that *Pasteuria* can effectively suppress nematode populations in natural fields and have promising potential as biocontrol agents. However, parasitism of nematodes by *Pasteuria* is generally highly species-, race-, and/or population-specific. A population of *Pasteuria* sp. was found on *Pratylenchus* sp. with a high percentage of nematodes having attached endospores in a soil sample collected from a field in Becker County in northwestern Minnesota, USA. The purpose of this study was to determine the ability of the *Pasteuria* population to parasitize different species and populations of *Pratylenchus*. Three populations (GR, WI, and PS) of *Pratylenchus penetrans* and one population each of *Pr. zeae*, *Pr. hexincisus*, *Pr. agilis*, *Pr. scribneri*, and *Pr. brachyurus* were tested for attachment of *Pasteuria* endospores to the nematodes. Field soil infested by *Pasteuria* and *Pratylenchus* was treated by freezing at –80 °C for 24 hours, presumably killing the nematodes while keeping the *Pasteuria* alive. The nematodes were obtained from gnotobiotic cultures on corn roots, and were added to the freeze-treated soil in vials. After 1 week of incubation at the room temperature (~23 °C), the mobile nematodes were recovered from the soil by incubating the soil on a screen immerged in water in a container for 24 hours, and the percentage of nematodes with attached spores were counted. Two populations (GR and WI) of *Pr. penetrans* had the highest percentage (55.5% and 46.5%, respectively) of individuals with attached spores, followed by *Pr. zeae* (26.2%). *Pr. hexincisus*, *Pr. agilis*, *Pr. brachyurus*, and *Pr. scribneri* had low percentage (1-3%) of nematodes with attached spores, while no spore was observed on the population PS of *Pr. penetrans*. This study demonstrated the species- and population-specificity of the *Pasteuria* population infecting species in the genus *Pratylenchus*.

EXPERIENCE WITH PASTEURIA ON SOYBEAN IN US. **Pedersen, P., D. Ireland, C. Watrin, and B. Battles.** Syngenta Crop Protection, 410 Swing Road, Greensboro, NC 27409, USA.

Soybean cyst nematode (*Heterodera glycines*) is the most destructive pathogen of soybean (*Glycines max* L.) throughout the World. Just in United States it costs the soybean producers more than $2 billion a year. *Heterodera glycines* is today found in most soybean producing areas of the world. Managing *H. glycines* is difficult but important, since when first found in a field, *H. glycines* cannot be eradicated. No single management tactic will help control it but the use of multiple management tactics can help minimize yield loss. Several management practices have been investigated to control this pest, such as the use of *H. glycines* - resistant cultivars, chemical control (nematicides), and the rotation with nonhost crops. Clariva Pn (*Pasteuria nishizawae*) seed treatment is a new offer (launched in 2014) from Syngenta that compliments the use of *H. glycines* - resistant cultivars. Pasteuria spp. are natural bacterial obligate parasites of nematodes with a unique mode of action. The Pasteuria spores (active ingredient) are highly effective and lethal to nematodes, without harming other organisms, plants and the environment. When delivered as a seed treatment, it will provide enhanced convenience and effectiveness for growers.

Pasteuria spores attach, penetrate and infect the nematode body, ultimately leading to its death. The technology starts to work immediately and reduces the reproductive rate even before killing the nematode. Pasteuria technology has the potential to be used across a broad range of crops. Data will be presented based on the last two years of experiences.

PHYLOGENY AND BIOGEOGRAPHY OF THE GENUS CEPHALENCHUS (NEMATODA: TYLENCHOMORPHA): EXPLORING MORPHOLOGICAL AND MOLECULAR CHARACTERS TO INFER SPECIES RELATIONSHIPS. **Pereira, T.[1], Q. Xue[2], K. Chang[1], M Mundo-Ocampo[3], and J.G. Baldwin[1].** [1]Department of Nematology, University of California, Riverside, CA 92521, USA; [2]Nematology Unit, Department of Biology, Ghent University, K.L. Ledeganckstraat 35, 9000 Ghent, Belgium; [3]CIIDIR-IPN, Unidad Sinaloa, Mexico.

Herein, conflicting views are revisited on the systematic position of the genus Cephalenchus (Tylenchidae vs. Tylodoridae) in relation to other tylenchs using populations sampled worldwide including from Brazil (BRA01, BRA02), Mexico (MEX), the United States (USA01, USA03, USA04, and USA05), Canada (CAN02), Vietnam (VIE01, VIE02), China (CHI01, CHI02), and Belgium (BEL01, BEL02). This broader sampling allows strongly testing hypothesis of monophyly and the validity of the genus while also providing additional insight into the biogeography of the group. Fourteen Cephalenchus populations were used for both morphological and molecular procedures. Furthermore, six additional populations previously deposited in the UCRNC as Cephalenchus sp. were morphologically identified from the United States (USA02, USA06, USA07, USA08), Canada (CAN01), and Thailand (THA). Morphological identification was based on morphometric analysis under LM and SEM observations of multiple individuals and by following published diagnostics. Molecular analyses were based on one mitochondrial (COI) and three ribosomal (18S, 28S, ITS) genes. Phylogenetic analyses (either combined or on a gene basis) always supported Cephalenchus as a monophyletic group and sister to Eutylenchus. On the other hand, the position of Cephalenchus within the tylench phylogenetic tree is still controversial or unresolved thus highlighting the importance of additional taxon sampling, particularly including additional genera believed to be closely related (e.g. Campbellenchus, Tylodorus). Hypothesis testing of alternative tree topologies for the placement of Cephalenchus (e.g. as part of Tylenchidae or its subfamilies) is rejected on the basis of two ribosomal (18S and 28S) genes. Within Cephalenchus, morphological characters such as amphid opening (lateral/longitudinal vs. dorso-ventral oriented) seem to be concurrent with molecular-based phylogenetic relationships, whereas the number of lines in the lateral field (six evolving to four lines) can be interpreted only as autapomorphic on one terminus. Additionally, MDS analysis based on morphometric data clear distinguishes short tail populations from medium-large tails. Moreover, SEM observations of these populations suggest that Cephalenchus species with a shorter tail might be congruent with these taxa also having a lateral/longitudinally-oriented amphid. Such quantitative morphological feature might be used to predict the amphid type and thus species relationships within the genus Cephalenchus.

EFFECTS OF VERMICOMPOST LEACHATE ON BACTERIA FEEDING NEMATODES. **Peterson, R. and S.P. Marahatta.** Kauai Community College, 3-1901 Kaumualiʻi Highway, Lihue, HI 96766.

Bacteria feeding nematodes can be used as an indicator of soil health. A strong presence of bacteria feeding nematodes in soil represents a solid foundation for the food pyramid to build upon, in which both plant and animal life can thrive. Vermicompost has been documented as a soil amending material for developing a healthy soil and enhancing beneficial soil microorganisms such as bacterivorous nematodes. Two laboratory trials were conducted to establish an optimal percent of vermicompost leachate required to enhance the population of bacteria feeding nematodes. Naturally occurring nematodes were extracted from cockroaches through the Baermann funnel technique. Sixteen 150-ml sized beakers were prepared by mixing nematode-containing water with vermicompost leachate at 0%, 5%, 10%, or 20% (v/v). The final volume of the mixture in each beaker was 20 ml. Beakers were arranged in randomized complete blocks with four replications and held at room temperature for 1 week. In Trial I, 10% and 20% leachate increased the number of bacteria feeding nematodes ($P < 0.05$). In Trial II, the number of bacteria feeding nematodes was higher only in the 20% leachate ($P < 0.05$). The lack of response of the 10% concentration in Trial II maybe correlated to a lower than average initial population. Nonetheless, the results were consistent in the 20% leachate. Thus, we concluded the higher the leachate solution, at least up to 20%, the greater the bacteria feeding nematode propagation. Farmers and researchers could use vermicompost leachate as a bacteria feeding nematode propagating medium.

KLEPTOPARASITIC NEMATODES OF DIPLOPODS IN NORTH AMERICA AND POTENTIAL PATHOGENIC EFFECTS. **Phillips, G.[1], E.C. Bernard[1], and R.M. Shelley[2].** [1]University of Tennessee, Entomology and Plant Pathology, 370 Plant Biotechnology Building, 2505 EJ Chapman Drive, Knoxville, TN 37996-4560; [2]Research Lab, North Carolina State Museum of Natural Sciences, MSC #1626, Raleigh, NC 27699-1626.

Nematodes of the order Oxyurida (Rhigonematida) are parasites of arthropods, including Diplopoda (millipeds). Species of Hethidae, Thelastomatidae *s. l.* and Rhigonematidae are commonly found in subtropical and tropical millipeds, but these nematodes are poorly known from temperate North American millipeds. In particular, Hethidae and *Coronostoma* spp. have not been observed in any indigenous North American millipeds, although *Heth mauriesi* has been described from the introduced milliped *Anadenobolus monilicornis* (Spirobolida: Rhinocricidae). *Coronostoma* spp. has not previously been

reported from North America and never from a spirobolidan milliped elsewhere in the world. Examination of the native Floridian spirobolidan, *Narceus gordanus* (Spirobolidae) produced numerous specimens of an undescribed *Heth* sp., as well as an undescribed species of *Coronostoma*. The presence of presumed indigenous (*Heth* sp.) and introduced species (*H. mauriesi*) raises the question of the susceptibility of indigenous millipeds to introduced nematodes. An experiment was conducted with nematode-free *Harpaphe haydeniana* (Polydesmida: Xystodesmidae) millipeds from Oregon and nematode-infested *N. americanus* and *Apheloria montana* (Polydesmida: Xystodesmidae) from Tennessee together in a lab culture. All individuals of *H.haydeniana* succumbed within three weeks of exposure to *N. americanus* and *A. montana*. Upon post-mortem dissection all *H. haydeniana* were infested with nematodes common to *N. americanus* and *A. montana*. On another occasion, a Tennessee milliped (*Euryurus leachii* (Gray, 1832) (Polydesmida: Euryuridae) apparently was infected with the undescribed *Heth* sp. after brief exposure to *N. gordanus*. These results suggest that introduced millipedes may be a threat to native species through their intestinal nematode faunas in addition to direct ecological competition.

THE MITOCHONDRIAL GENOME OF *GLOBODERA ELLINGTONAE* IS DIVIDED INTO TWO CIRCLES WITH BOTH IDENTICAL AND UNIQUE SEQUENCE REGIONS. **Phillips, W.S.[1], A.M.V. Brown[2], D.K. Howe[2], A.B. Peetz[1], D.R. Denver[2], and I.A. Zasada[1].** [1]USDA-ARS Horticultural Crops Research Laboratory, 3420 NW Orchard Avenue, Corvallis, OR 97330; [2]Department of Integrative Biology, 3029 Cordley Hall, Oregon State University, Corvallis, OR 97331.

The potato cyst nematodes (PCN), *Globodera pallida* and *G. rostochiensis*, have unusually structured mitochondrial genomes consisting of multiple different ''mini-circles.'' Such multiparitite mitochondrial genomes are extremely rare in animal genomes, having been found in a few species of lice and box jellyfish but in no other genera of nematodes. We are assembling the genome of a newly discovered *Globodera* species, *G. ellingtonae*, which is closely related to PCN. Using 300 base pair MiSeq reads and PacBio reads averaging approximately 2.5 kilobases (kb) generated for the whole genome assembly, we have assembled the mitochondrial genome of *G. ellingtonae*. The mitochondrial DNA of *G. ellingtonae* assembled into two circles: one that is 14.6 kb in length and the other 17.6 kb in length, each with different mitochondrial genes. Given that *G. pallida* has been shown to have at least six different mitochondrial mini-circles, the largest being 9.5 kb in length, the finding of two larger circles in *G. ellingtonae* was unexpected. The assembly from next generation sequence has been confirmed by PCR amplification, cloning, and Sanger sequencing of both circles as well as by Southern blots. The circles each contain distinct mitochondrial genes but have a shared, highly similar sequence region of approximately 6.8 kb not containing any mitochondrial genes and with unknown function. The shared region does have a marked difference in base composition, with the proportion of thymine bases dropping to 40 to 42% in the shared region as compared to 50% in the non-shared coding sequence regions. Interestingly, at least at the population level, evidence from next generation sequence and Southern blots indicates the smaller circle is present at approximately four fold higher copy number than the larger circle. As with the other animals in which a multipartite mitochondrial genome has been found, the functional significance of this arrangment is unknown.

TEMPERATURE AND DEVELOPMENTAL DYNAMICS OF *GLOBODERA ELLINGTONAE*. **Phillips, W.S., S.R. Kieran, M. Kitner, and I.A. Zasada.** USDA-ARS Horticultural Crops Research Laboratory, 3420 NW Orchard Avenue, Corvallis, OR 97330.

A new *Globodera* nematode was discovered in 2008 in Oregon and Idaho and was described as a new species, *G. ellingtonae*, in 2012. Morphological and DNA evidence indicate it is closely related to the potato cyst nematodes (PCN), *G. pallida* and *G. rostochiensis*, as well as the tobacco cyst nematode *G. tabacum*. We have tracked the development of this nematode in potato roots grown at different temperatures, ranging between 10 and 26.5 °C, in growth chambers as well as over two seasons in the Oregon field from which this nematode was originally discovered. The time until the peak population for each developmental stage in growth chamber experiments decreased between temperatures 10 to 20 °C. At 20, 23, and 26.5 °C the times to each peak developmental stage were fairly similar between temperatures, but the duration of each stage in roots tended to decrease with increasing temperature. In both the field and growth chamber experiments, there appeared to be at least two separate waves of root invasion by second-stage juveniles (J2) within the first five weeks after planting. In both years of field experiments, we observed an additional late season hatch of J2, presumably from eggs produced in that growing season, beginning 12 to 13 weeks after planting; however, very few of these J2 successfully invaded the remaining potato roots. Based on our data, we predict *G. ellingtonae* could complete its life cycle within a four month growing season when soil temperature averages are as low as 13 °C and within 2.5 months when temperature are higher than 17 °C.

COMPARISON OF TWO NEMATODE EXTRACTION TECHNIQUES. **Plaisance, A. and G. Yan.** North Dakota State University, Department of Plant Pathology, Fargo, ND 58108.

Plant parasitic nematodes (PPN) are an important group of pests that affect productivity of many crops. Limited information exists on the occurrence of nematodes on crops in North Dakota, making methods of rapid extraction and identification of samples important. Samples from a nematode survey conducted in 2014 on six field crops (corn, wheat, soybean, barley, potato, pea) were selected to compare results of nematode extraction via a rapid

centrifugal-floatation technique using sugar for separating nematodes from soil and the Whitehead and Hemming tray method, and to compare with the results of extractions from a commercial laboratory. Of the 28 samples selected, pin (*Paratylenchus* spp.), lesion (*Pratylenchus* spp.), foliar (*Aphelenchus* spp. and *Aphelenchoides* spp.), spiral (*Helicotylenchus* spp.), stunt (*Tylenchorhynchus* spp.), dagger (*Xiphinema* spp.), and stubby-root (*Trichodorus* spp. and *Paratrichodorus* spp.) nematodes were detected in final nematode suspensions. Densities of the nematodes per 200g of soil averaged 62 (pin), 86 (lesion), 44 (foliar), 471 (spiral), 88 (stunt), 6 (dagger), and 0 (stubby-root) using the sugar method and 60 (pin), 74 (lesion), 133 (foliar), 322 (spiral), 39 (stunt), 8 (dagger), and 24 (stubby-root) using the tray method. Analysis of each genus and nematode category (total ectoparasitic PPN and total PPN) showed that there were significantly more foliar nematodes extracted from the tray method than from the sugar method, but there were no significant differences between the methods for any other genera or categories. Overall, the sugar floatation method successfully extracted 13% more total PPN. Numerically, more PPN of each genus and category were recovered from the sugar method than from the tray method, with the exception of stubby-root and foliar nematodes. Lastly, although the sugar and tray methods recovered approximately double the amount of total spiral and lesion nematodes than a commercial laboratory, there were no statistically significant differences between the findings of these two methods and the findings of the commercial laboratory. In the summer of 2015, more samples will be collected and PPN will be extracted using both methods to further determine the most efficient form of extraction. Furthermore, the soil texture of each sample could impact the efficiency of each method of extraction.

CRICONEMATIDS AS A MODEL FOR UNDERSTANDING THE CHALLENGES OF HOMOPLASY IN PHYLOGENY, SPECIES DETERMINATIONS, CLASSIFICATION AND BIOGEOGRAPHY. **Powers, T.O.** Department of Plant Pathology, University of Nebraska-Lincoln, Lincoln, NE 68583-0722.

The conserved morphological structure of nematodes has strongly influenced our interpretation of nematode distribution, biogeography, and classification. Difficulties in assessing species boundaries when solely using morphological characters has led to the recognition of a disproportionate number of cosmopolitan species. These widespread distributions are particularly hard to reconcile when the nematode species do not appear to possess specialized dispersal or survival strategies. To address questions of nematode biogeography and biodiversity we have initiated an integrated approach using COI barcoding of individual specimens in the suborder Criconematina. The criconematids are well-suited for a biogeographic study due to their global distribution, abundance in native plant communities, and the array of easy to measure cuticular structures that figure prominently in their classification. To date over 1,100 fully analysed and annotated specimens exist on a phylogenetic tree that serves as a biological framework for systematic analysis. This tree, together with analyses of population structure lead to the following conclusions. Cryptic species, as evidenced by genetically distinct lineages within a single morphologically defined species, are common. Many of our morphologically recognized species like *Mesocriconema xenoplax*, *Crossonema menzeli*, *Criconema permistum*, and *Mesocriconema curvatum* are actually species complexes. Many nematode lineages, especially those in native plant communities, display geographically structured patterns. Nematodes from formerly glaciated, northern latitudes in North America, have lower haplotype diversity than southern isolates. Evidence of long distance dispersal is rare, except in agricultural ecosystems. Genetically distinct lineages, when analysed by discriminant function analysis, often reveal a discrete morphological signal. This morphological-molecular correspondence supports the hypothesis that distinct haplotype lineages recognized by COI barcoding represent independent evolutionary lineages and therefore warrant species status.

PARAPHYLY OF THE GENUS DITYLENCHUS FILIPJEV (NEMATODA: TYLENCHIDA) CORRESPONDING TO THE D. TRIFORMIS-GROUP AND THE D. DIPSACI-GROUP MORPHOLOGICAL. **Qiao, Y.[1], Q. Yu[2], A. Badiss[2], M.A. Zaidi[2], E. Ponomareva[2], Y. Hu[3], and W. Ye[4].** [1]College of Agriculture, Shanxi Agriculture University, Tangu, Shanxi Province, China; [2]Eastern Cereal and Oilseed Research Centre, Agriculture and Agri-Food Canada, Ottawa, Ontario, Canada; [3]China Agriculture University, Beijing, China; [4]Nematode Assay Section, Agronomic Division, North Carolina Department of Agriculture & Consumer Services, NC.

The genus *Ditylenchus* historically has been divided into two groups, the *D. triformis*-group and the *D. dipsaci*-group, based on morphological and biological characters. Eighteen populations belonging to five species of *Ditylenchus* were studied, *D. africanus, D. destructor, D. myceliophagus, D. dipsaci* and *D. weischeri*, the first three species representing the *D. triformis*-group and the last two the *D. dipsaci*-group. Nematodes of the *D. triformis*-group were cultured on fungi and those of the *D. dispaci*-group on excised roots of plant hosts. DNA sequences of regions of the nuclear ribosomal first internal transcribed spacer (ITS1) and the small subunit 18S were employed in phylogenetic analyses which included sequences of closely related species from Genbank. Randomly amplified polymorphisms of genomic DNA (RAPDs) were also studied. Two clusters or clades corresponding to the two *Ditylenchus* groups were consistently observed with significant statistical support from all three datasets. The phylogenetic analyses revealed that the genus *Ditylenchus*, as originally determined by morphology, is paraphyletic with the two groups separated by species of *Anguina* and *Subanguina*.

THE SPATIAL DISTRIBUTION OF NEMATODE TAXA IN ARABLE AND (SEMI-) NATURAL FIELDS. **Quist, C.[1], P. Mooijman[1], D. Brus[2], G. Gort[3], S. van den Elsen[1], C. Mulder[4], A. Termoshuizen[5], J. Bakker[1], and J. Helder[1].** [1]Laboratory of Nematology, Wageningen University, P.O. Box 8123, 6700 ES, Wageningen, The Netherlands; [2]WUR-Alterra, Soil Geography, Droevendaalsesteeg 3, 6708 PB Wageningen, The Netherlands; [3]Biometris, Dept. Plant Sciences, Wageningen University and Research Centre (WUR), Droevendaalsesteeg 1, 6708 PB, Wageningen, The Netherlands; [4]National Institute for Public Health and the Environment (RIVM), A. van Leeuwenhoeklaan 9, 3720 BA Bilthoven, The Netherlands; [5]SoilCares Research, Binnenhaven 5, 6709 PD Wageningen, Netherlands.

Nematodes are abundant and diverse in virtually any soil, and their communities have representatives in all trophic layers of the soil food web. Therefore nematodes have a potential as a proxy for the biological condition of soils and sediments. So far this potential is underexploited, mainly for technical reasons; the microscopic analysis of nematode assemblages is labour intensive and requires extensive expertise. With the availability of a relatively large molecular framework, it became possible to design molecular assays that allow for the quantitative analysis of individual taxa against complex DNA backgrounds (Vervoort et al. 2012, Rybarcyzk et al. 2012).

To determine the biological condition of soil at landscape scale, detailed insight in the spatial distribution of nematode taxa is essential. Information about distribution patterns for individual nematode species, genera or families at hectare scale and above would allow for sampling strategies with predictable accuracies. However, this is easier said than done. The availability of a range of quantitative (q) PCR assays to detect and quantify nematode assemblages, made it possible to investigate the spatial distribution of nematode taxa at hectare level, an undertaking that requires the analyses of large number of sampling plots. In order to assess the degree of spatial variability of individual nematode taxa across the Netherlands 12 fields of a single hectare each were sampled intensively. Using a sampling grid optimized for geostatistic analysis, composite samples were taken from 96 - 116 sampling plots per field. All samples ($n = \sim 1200$) were analysed with 25 - 32 qPCR assays, to measure total nematode densities, densities of individual nematode taxa and one internal control. In total about 35,000 qPCR reactions were run, results – visualized as semi-variograms and surface maps – show the distribution patterns. The data that will be presented gives insight into distribution patterns of multiple free-living and plant-parasitic nematode taxa in different soil types and in arable and semi-natural systems. Results are essential ingredients for the design of scientifically sound sampling schemes at landscape scale.

INFERRING PROCESSES OF EVOLUTIONARY DIVERSIFICATION ASSOCIATED WITH DEVELOPMENTAL PLASTICITY IN DIPLOGASTRID NEMATODES. **Ragsdale, E.J.** Department of Biology, Indiana University, 915 E. 3rd Street, Bloomington, IN 47405.

The ability of a genetic program to produce variable phenotypes – that is, developmental plasticity – may be a potent accelerator of evolutionary transitions. Numerous case studies in animals and plants show a link between plasticity and adaptability, and plastic traits often mirror phenotypic differences among species. Developmental plasticity may therefore be an important generator of phylogenetic patterns in some taxa. However, an integration of historical and functional evidence is still needed to test this principle. Nematodes of Diplogastridae, some of which produce alternative feeding morphotypes in response to different environmental cues, are an ideal system for investigating the relationship between plasticity, specifically polyphenism, and phenotypic diversification. With respect to their closest outgroups, Diplogastridae are demonstrably diverse in their feeding morphologies. As inferred from a solid phylogenetic infrastructure, this diversity correlates with the historical presence of polyphenism. On a microevolutionary scale, studies of populations of polyphenic species can explore divergence of plasticity regulation, which is predicted to change with local environmental differences. Using a model organism for developmental genetics, the diplogastrid *Pristionchus pacificus*, genetic regulators of polyphenism can be identified and examined across populations and close species. Work on *P. pacficus* has shown the feasibility of such a program: a developmental switch discovered in this species was found to diverge with plasticity phenotypes in wild populations, and it can be likewise tested for function in other species. Thus, the integration of phylogenetics and functional genetics in a model nematode system offers a promising approach to explore the contribution of developmental plasticity to evolutionary pattern and process.

BUILDING UP THE TEAM FOR INTERDISCIPLINARY TEAM SCIENCE. **Read, E.[1], M. O'Rourke[2], G.S. Hong[3], P.C. Hanson[3], L.A. Winslow[3], S. Crowley[4], C.A. Brewer[5], and K.C. Weathers[6].** [1]Center for Integrated Data Analytics, U.S. Geological Survey, Middleton, Wisconsin, 53562; [2]Department of Philosophy, and AgBioResearch, Michigan State University, East Lansing, Michigan, 48824; [3]Center for Limnology, University of Wisconsin-Madison, Madison, Wisconsin, 53706; [4]Philosophy Department, Boise State University, Boise, Idaho, 83725; [5]Department of Biological Sciences, University of Montana, Missoula, Montana, 59812; [6]Cary Institute of Ecosystem Studies, Millbrook, New York, 13545.

For scientists to solve the complex and large-scale challenges that face society, the ability to exchange research-relevant information and develop collaborative relationships that cross disciplinary boundaries is essential, but these communication skills are rarely taught. We developed a set of modular, adaptable training components designed to increase the potential of scientists to engage in information exchange and relationship development in interdisciplinary team science settings. A pilot of

this program was developed by a leader in ecological network science, the Global Lake Ecological Observatory Network (GLEON), and was initially designed for early career scientists, but is relevant to scientists at any career stage. Results of the pilot indicate that the training resulted in improvement in scientists' confidence in team-based science collaborations. Participants in the program navigated human-network challenges, improved communication skills, and increased their ability to build trusting professional relationships, all in the context of producing collaborative scientific outcomes. We describe the rationale for the key training elements, and provide evidence that the training is effective in building essential team science skills.

MODELING THE IMPACT OF WEATHER ON WINTER SURVIVAL OF *MELOIDOGYNE HAPLA* IN QUEBEC, CANADA. **Ricard, M-P., G. Bélair, and G. Bourgeois.** Agriculture and Agri-Food Canada, Saint-Jean-sur-Richelieu, Quebec CANADA J3B 3E6.

Weather factors suspected to have a significant effect on the field winter survival of *M. hapla* were investigated using a 9-year data set of fall and spring nematode population densities. The objective was to develop a bioclimatic model that will provide an estimate of spring population levels as a function of winter weather conditions. Spring and fall nematode densities of each year were estimated by both a galling bioassay method and the Baermann pan method. The rates of population change from fall to spring were calculated in order to determine the survival rate of *M. hapla* after each winter on the basis of the weather data collected during those winters. Relative rates of density change varied from year to year and also with the method of assessing nematode densities. Stepwise regression was used to find the most comprehensive model that best expressed the relationship between the various factors and the relative rates of change in nematode winter density. Two significant weather factors were retained: the quantity of rainfall during the 5 days prior to the first occurrence of soil temperature ≤ 0°C, which usually occurs at the beginning of December, and the number of degrees ≤ -2°C during the month of December. Rainfall just before ground frost was found as the main factor influencing on *M. hapla* survival rate. The galling bioassay method provided the most reliable assessment of winter survival which accounts for both the mobile J_2 and the egg masses in the soil. The model implies that the two weather factors have a positive effect on the survival of J_2 and a negative effect on egg masses in the soil.

IN VITRO COMPARISON OF LITCHI TOMATO AND POTATO DIFFUSATE ON HATCHING OF *GLOBODERA PALLIDA*. **Rowley, J. and L.M. Dandurand.** Plant, Soil, and Entomological Sciences, University of Idaho, Moscow, Idaho 83844.

The aim of this study was to determine the optimum time point during a plant's growth for stimulation of *Globodera pallida* hatching. Diffusates from either potato or the trap crop *Solanum sisymbriifolium* (litchi tomato, LT) were collected at different times: 2, 4, or 6 weeks after planting. Hatching of *Globodera pallida* induced by root diffusates obtained at different times and in different concentrations was determined *in vitro*. Root diffusates were collected from potato and LT at 2, 4, and 6 weeks. Cysts of *G. pallida* were gently crushed and eggs were submerged in diffusates for two weeks. Eggs and juveniles (J2 stage) were counted at the start of the experiment, and after one and two weeks. To determine the activity of hatching stimulus from either potato or LT, diffusates collected at each time point were further characterized by assessing hatch of eggs exposed to the following concentrations: 1:1, 1:10, 1:100 and 1:1000. Differences were seen between the two plant types, and at different times and diffusate concentrations. The more concentrated diffusate collected from litchi tomato, at all time points, stimulated hatching more than diffusate collected from potato. However, hatching was stimulated by potato root diffusate even at the lowest concentration tested, 1:1000, whereas LT diffusate did not stimulate hatch at that concentration. In addition, diffusate collected after 4 and 6 weeks stimulated hatch more than diffusate collected after 2 weeks, for both plant species.

STUDY OF RING NEMATODE SENSITIVITY TO GGI EXP 15. **Sances, F.[1], B.A. Aglave[1], B. Booker[1], and G. Nijak[2].** [1]Florida Ag Research, 3001 N. Kingsway Road, Thonotosassa, FL 33592; [2]Green and Grow, Inc. Bldg. #2, 400 Josephine St, Austin, TX 78704.

This study was conducted to evaluate the sensitivity of ring nematodes to GGI EXP 15 which is a multi-strain fermentation culture produced by Green and Grow, Inc.

Egg Hatching Study: Solutions of GGI EXP 15 in three different concentrations were prepared. Ring nematode (*Criconemella sp.*) eggs were added to the solutions and the number of eggs that hatched was determined after ten days. Approximately 30% fewer eggs hatched in the GGI EXP 15 solution at 1% v/v than hatched in the untreated control. Lower concentrations (0.1% and 0.01%) were not found to be effective.

Juvenile Nematode Mortality: Solutions of GGI EXP 15 were prepared in three concentrations. Ring nematode (*Criconemella sp.*) juveniles were added to the solutions in a well plate. At 72 hours after the application the GGI EXP 15 at 1% v/v showed a statistically significant higher mortality than the other treatments (no differences found at 24 and 48 hours). It should be noted that the majority of the nematodes in this treatment were caught in a globule of material thought to be associated with the GGI EXP 15 and were unable to swim freely. Possibly this restriction of movement caused the mortality of the nematode juveniles. It is believed that this material is a primary metabolite of the fermentation culture that has chelator like properties.

DETECTION AND DENSITY OF POTATO CYST NEMATODES IN THE NILGIRI HILLS OF SOUTH INDIA, INDIA. **Saranya C.[1], P. Sundararaj[2], and S.L. Hafez[2].** [1]Bharatiar University, Coimbatore-641046, Tamilnadu, India; [2]University of Idaho Parma REC, 29603 U of I Lane, Parma, ID 83660, USA.

Potato cyst nematodes (*Globodera pallida and Globodera rostochiensis*) are the major nematode pests causing 80 percent yield loss on potato crop in the Nilgiri hills of Tamilnadu, India. In 2013, soil samples were collected in all 13 potato growing areas of Nilgiris district. A total of 130 soil samples were collected from the potato field. Maximum cyst population of *G. pallida was* observed in Kagguchi village (100) while the minimum was observed in Kadanadu (1). Maximum and minimum cyst count of *G.rostochiensis* was recorded in Balacola (64) and Kagguchi (1) village respectively. Maximum frequency of occurrence of both species was 9.2% in Nanjanad and Sholur. Continuous monoculture of potato and cropping system pattern are the major factors that increased the population density of both cyst nematode species in Nilgiri hills.

BIOLOGICAL AND MOLECULAR STUDIES WITHIN TRIPARTITE INTERACTIONS BETWEEN PLANT PARASITIC NEMATODES, BENEFICIAL ENDOPHYTES AND PLANTS. **Schouten, A.** INRES-Molecular Phytomedicine, University of Bonn, Karlrobert Kreiten Strasse 13, 53115 Bonn, Germany. aschout@uni-bonn.de

Plant associated fungi can affect plant-nematode interactions. Over the past years, we have focussed on particular endophytic root associated *Fusarium oxysporum* isolates, which trigger a systemic resistance towards both sedentary and burrowing nematodes in various plant species, like tomato, rice and banana. The presence of the endophyte has a negative effect on both the infection of plants by nematodes and the development of the nematodes that still succeed in the infection process. Remarkably, although colonization of the endophyte is restricted to the root system, the beneficial effect of the same endophyte can also be found in the leaves by negatively affecting herbivorous and omnivorous insects. These observations lead to ample speculation on how the endophyte can trigger defense responses in the plant and whether or not the endophyte can also directly affect nematode infection and development. Biological and molecular studies in tomato and Arabidopsis are now in progress to further dissect and pinpoint the mechanisms leading to the observed effects. This knowledge can be useful for quickly validating new and more effective endophytes for controlling nematode infections.

SYNERGISM OF THE ROOT-KNOT NEMATODE FOR FUSARIUM WILT IN CUCURBITACEOUS PLANTS. **Seo, Y. and Y-H. Kim.** Department of Agricultural Biotechnology and Research Institute of Agriculture and Life Sciences, Seoul National University, Seoul 151-921, Korea.

The fusarium wilt of the oriental melon is still prevalent in major oriental melon-growing areas although a resistant cultivar shintozoa (*Cucurbit maxima* x *C. moschata*) has been widely used as a stock plant for grafting oriental melon cultivars. The potential reason for the current disease prevalence was examined based on two instances; the occurrence of new causal agents pathogenic to shintozoa and increase of the disease severity with the co-infection of the root-knot nematode that increases the disease severity synergistically with the fusarium wilt disease. For this, twenty-nine *Fusarium* isolates obtained from oriental melon fields were inoculated on shintozoa, cucumber and oriental melon, in which most *Fusarium* isolates were more virulent to oriental melon and cucumber than shintozoa. Two representative isolates *F. oxysporum* F5 *and F. proliferatum* F6 were selected and inoculated on the plants alone or in combination with the root-knot nematode (*Meloidogyne incognita*) at high and low inoculum concentrations. In this test, both *Fusarium* species produced severer wilt symptoms in combination with *M. incognita* than the fungal isolates alone, in which F6 caused more extensive destruction of vascular tissues with poor giant cell formation than F5. Root gall formation and development of giant cells were decreased in the plants co-inoculated with both fungi and nematode, compared with *M. incognita* alone, more with F6 than F5. All of these results suggest that the current prevalence of the fusarium wilt on oriental melon fields in Korea may be potentially related to its increased disease severity caused by the synergism of the root-knot nematode, although the occurrence of new fusarium isolates virulent to shintozoa is not to be totally exempted from one of the potential reasons.

PROGRESS TOWARDS A *ROTYLENCHULUS RENIFORMIS* GENOME ASSEMBLY. **Showmaker, K.C.[1,2], W.S. Sanders[1,3], M.A. Arick II[1], Z.V. Magbanua[1], D.G. Peterson[1,4], and M.J. Wubben[2,4,5].** [1]Institute for Genomics, Biocomputing, and Biotechnology, Mississippi State University, Mississippi State, MS 39762; [2]Department of Biochemistry, Molecular Biology, Entomology and Plant Pathology, Mississippi State University, Mississippi State, MS 39762; [3]Department of Computer Science and Engineering, Mississippi State University, MS; [4]Department of Plant and Soil Sciences, Mississippi State University, Mississippi State, MS 39762; [5]USDA-ARS, Genetics & Precision Agriculture Research Unit, Mississippi State, MS 39762.

Rotylenchulus reniformis, commonly known as the reniform nematode, is a polyphagous plant parasitic nematode parasitizing at least 314 plant species including crops such as *Gossypium hirsutum* (cotton), *Glycine max* (soybean), and *Ipomoea batatas* (sweet potato). To better understand reniform biology and its adaption to a semi-endoparasititstic lifestyle we have sequenced and annotated the reniform nematode genome. The sequencing strategy included Illumina Hiseq, MiSeq, and GAIIx sequencing platforms coupled with Illumina traditional, PCR Free and Mate Pair libraries. DNA for sequencing was derived from a pooled population of reniform eggs and via whole genome amplification of single nematodes. Furthermore, RNAseq data from 5 developmental lifestages, i.e., egg, second stage juvenile (J2), third stage juvenile (J3), vermiform adult (adult), and

sedentary female (SF), was generated to assist in structural genome annotation and protein prediction. The draft genome was assembled with the ABySS assembly algorithm into 5,584 scaffolds larger than 10,000 bp summing to 195.9 Mb with a scaffold N50 of 47,721 bp. Genomic structural and functional annotations include: repeat characterization, non-coding RNA prediction, protein prediction and annotation, and pathway annotation. Comparative genomic analyses were conducted with the previously sequenced plant parasitic nematodes *Meloidogyne hapla*, *M. incognita*, *Globodera pallida* in addition to the free-living model species *Caenorhabditis elegans*. Core Eukaryotic Genes Mapping Approach (CEGMA) showed that the draft reniform assembly presented here was comparable to the completeness of previously assembled plant parasitic nematode genomes. RNAseq and shotgun proteomics provide evidence for the transcription and translation for predicted proteins. This study provides a valuable resource, to the research community that can be used to further investigate reniform nematode biology and adaption to its environment.

PLANT HEALTH PROMOTING RHIZOBACTERIA (PHPR) AND PLANT PARASITIC NEMATODES: MODES-OF-ACTION AND INTERRELATIONSHIPS WITH ENDOPHYTIC FUNGAL ANTAGONISTS. **Sikora, R.A.[1] and A. Martinuz[2].** [1]INRES-Phytomedizin, Karlrobert-Kreiten-Str. 13, 53115 Bonn, Germany; [2]Program Manager, Disaster Risk and Climate Change (PAGRICC), Ministry of Environment and Natural Resources of Nicaragua.

Extensive research has been conducted on the interrelationships between Plant Health Promoting Rhizobacteria and plant parasitic nematodes. This review will look at the different antagonistic activities of root-associated bacteria on the root-knot nematode, *Meloidogyne incognita,* and the potato cyst nematode, *Globodera pallida*, in tomato and potato, respectively. The bacteria discussed here are those that: (1) have the ability to colonise both the rhizosphere and the endorhiza at some point in their life-cycle; (2) can grow saprophytically in the soil or in the rhizosphere; and (3) have plant health promoting activity. The mechanism of action, including effects on attraction, nematode development and the involvement of induced resistance on nematode behavior will be outlined. The influence of PHPR on endo-mycorrhiza and mutualistic endophytic fungi that reduce nematode infection will also be presented. The importance of seed and seedling inoculation with plant health promoting rhizobacteria will be discussed.

DETECTION AND IDENTIFICATION OF *ANGUINA FUNESTA, A. AGROSTIS, A. TRITICI*, AND *A. PACIFICAE* BY REAL-TIME PCR. **Skantar, A.M.[1], W. Li[2], Z. Yan[2], and M.K. Nakhla[2].** Nematology Laboratory, USDA Agricultural Research Service, Beltsville, MD 20705; United States Department of Agriculture–Animal and Plant Health Inspection Service, Center for Plant Health Science and Technology, Beltsville Laboratory, (USDA-APHIS-PPQ-CPHST Beltsville Laboratory), Beltsville, MD 20705.

Several seed, leaf, and stem gall nematodes are of significance to the forage and landscape grass and livestock industries. In North America, the bentgrass nematode, *Anguina agrostis*, reduces seed production on *Agrostis tenuis* and several other grass species. *Anguina funesta* is a seed-gall nematode that is most significant for its association with the toxigenic bacteria *Rathayibacter toxicus*. The wheat seed gall nematode *Anguina tritici* causes significant damage to wheat and other cereals; while it has been found in many countries worldwide, it has not been detected in the United States since 1975. The stem gall nematode *Anguina pacificae* is the most devastating pest of *Poa annua* putting greens in Northern California golf courses; it poses a major threat to the California golf industry, which supports 160,000 jobs and annually contributes more than $6 billion directly to the economy. Molecular methods based upon sequence variation in the ribosomal internal spacer region (ITS) are useful for accurate identification of *Anguina* species. We present new species-specific primers and TaqMan probes for real-time PCR identification of *Anguina agrostis, A. funesta, A. tritici,* and *A. pacificae*. Primer and probe combinations were shown to be specific for the intended species and were sensitive enough to detect 10 copies of nematode ribosomal DNA. PCR was also specific and sensitive in duplex assays that included genus-specific internal control primers as well as species-specific primers and probes. These standardized real-time PCR protocols should facilitate fast and accurate identification of *Anguina* species by diagnostic laboratories.

NEMATODES THE HIDDEN ENEMIES: TOWARDS AN INTEGRATED CONTROL SOLUTION FOR THE SUGAR BEET CYST NEMATODE *HETERODERA SCHACHTII*. **Slaats, B.[1], D. Belles[2], and P. Pedersen[3].** [1]Syngenta Crop Protection AG, Schaffhauserstr 101, 4332 Stein AG, Switzerland; [2]Syngenta Crop Protection LLC, 4037 E. Karsten Dr., Chandler, AZ 85249, USA; [3]Syngenta Crop Protection LLC, 317 330th Street, Stanton, MN 55018, USA.

Recent trends in farming practices have increased nematode pressure. While traditional techniques to control nematodes (e.g. fumigation) are being banned, growers have developed better awareness and now seek modern solutions for nematode control. Syngenta has built a leading position in nematode control with a portfolio offering growers a range of solutions based on a complementary offer in Traits, Crop Protection and Biologicals. Avicta® is a seed-applied nematicide solution based on the active ingredient Abamectin, which was first launched in 2006 and has now been registered for several crops in the Americas. Clariva® (*Pasteuria nishizawae*) is a bionematicide, which came from the acquisition of Pasteuria Biosciences in 2012; it was first registered in the USA for control of *Heterodera glycines* in soybean in 2013. Its use was recently extended to sugar beet as sugar beet cyst nematodes (*Heterodera schachtii*) are an increasing problem in sugar beet production areas

and can cause yield loss of up to 60% depending on the level of nematode infestation. Although lower levels of nematode infestation may not cause visible symptoms of damage, without implemented control strategies a build-up of the population will begin. Great efforts of sugar beet breeders over the past decade have produced new beet cyst tolerant varieties which allow for cost-effective yields from nematode infested fields. These varieties have however little impact on nematode populations. Resistant varieties on the other hand also reduce the population of nematodes, however currently this trait comes at the expense of yield. Tolerant varieties ensure good yield under infected and un-infected conditions (whether nematode infection is in patches or across the entire field). Clariva® seed treatment compliments the use of nematode tolerant cultivars to manage *H. schactii*. *Pasteuria nishizawae* is natural bacterial obligate parasite of nematodes with a unique mode of action. The *Pasteuria* spores (active ingredient) are highly selective to nematodes; the individual strains are very specific to a nematode species or genus. *Pasteuria* spores attach, penetrate and infect the nematode body, strongly reducing the reproductive rate of the female before ultimately leading to its death. In small plot sugar beet field trials with moderate- to high-levels of *H. schactii* in Idaho, Colorado, and Michigan seed-applied Clariva® increased the sugar yield per acre versus the insecticide/fungicide check.

PRISTIONCHUS SCRATCHPADS; A NEW FRAMEWORK FOR MODERNIZING SYSTEMATICS. **Sommer R.J. and M. Herrmann.** Max-Planck Institute for Developmental Biology, D-72076 Tübingen, Germany; Sommerlab.org; www.pristionchus-sp.de

Our lab has developed the nematode *Pristionchus pacificus* as a model system in evolutionary and comparative biology. Our work integrates developmental biology with ecology and population genetics combining lab-based mechanistic studies in genetics and molecular biology and fieldwork. Such work requires a detailed natural history and phylogenic perspective. Over the years, we have collected and described a total of 28 *Pristionchus* species, the majority of which lives in an association with scarab beetles, similar to what is found for *P. pacificus*. Like in most nematode genera, information on the taxonomy, systematics and phylogeny is spread over many sources and is often unavailable to individual researchers.

We describe here *Pristionchus*-Scratchpads, which we developed as a dynamic, searchable and community-driven online platform. *Pristionchus*-Scratchpads covers Pristionchus taxonomic data in morphology, molecular diagnosis and biogeography and provides links to the published literature. Thus, *Pristionchus*-Scratchpads helps researchers throughout the world obtain an overview of published work on *Pristionchus* and serves as a platform for requesting live material. We will discuss *Pristionchus*-Scratchpads as a framework for similar projects in other nematodes and will describe the necessary boundary conditions. Also, we want to discuss future taxonomic research in *Pristionchus* itself.

DETECTING GENOTYPIC VARIATION AMONG THE SINGLE SPORE ISOLATES OF *PASTEURIA PENETRANS* POPULATION OCCURRING IN FLORIDA USING SNP-BASED MARKERS. **Soumi, J.[1], L.M. Schmidt[2], P. Timper[3], T. Hewlett[2], C. Watrin[2], and T. Mekete[1].** [1]University of Florida, Entomology and Nematology Department; [2]Syngenta Crop Protection, LLC; [3]USDA ARS, P.O. Box 748, Tifton, GA 31793.

Pasteuria penetrans is a naturally occurring soil-borne endospore-forming bacterium, which functions as a castrating parasite of plant-parasitic nematodes belonging to the genus *Meloidogyne*. *Pasteuria penetrans* is established as an effective biological control agent for control and management of root knot nematodes (RKN). Previous studies have suggested that field populations of *P. penetrans* are heterogeneous and also suggest that individuals within a given population vary in degree of host specificity and virulence towards the same host. This study examined genotypic variation and virulence characteristics of six clonal *P. penetrans* spore lines produced though single spore infections of individual host nematodes exposed to a field population of *P. penetrans* isolated from *Meloidogyne arenari*a in Florida. Genetic variability of six clonal lines of *P. penetrans* was assessed based upon sequence analysis of six protein-coding genes and the 16S rRNA gene. The results showed an average of one SNP for every 69 bp in the 16S rRNA partial cds while protein-coding sequences did not show any variation among the clonal lines. Hierarchical cluster analysis of 16S rRNA sequences placed the clones into three groups: Clonal lines 16ssp, 30ssp, 25ssp and 26ssp clustered together and separate from the distinct clonal lines 17ssp and 18ssp. Evaluation of the clonal lines ability to attach and parasitize different species of RKN and populations obtained from different geographic locations resulted in significant differences in spore attachment and virulence amongst the clones. Clonal lines 16ssp and 30ssp provided the highest rates of spore attachment (24-40spores/juvenile) and were positive for infectivity in all RKN species tested, while 18ssp provided the lowest attachment rate (1-11spores/juvenile) and lowest level of spore production on *M. arenaria*. In summary, our study demonstrated that a field population of *P. penetrans* is highly heterogeneous with regard to homology in the 16S rRNA gene and individual members demonstrate varying levels of host virulence. For example 16ssp and 30ssp demonstrated a wide host range and robust virulence among RKN species, while 18ssp demonstrated a tighter host range and lower overall virulence. Our study demonstrates that the SNP marker based on the variable region (5' end) of 16S rRNA region gives sufficient resolution to discriminate single spore isolates of *P. penetrans* and has the potential to be developed as a tool for discriminating host specificity and virulence.

IMPORTANCE OF GLIA-SECRETED MATRIX OF NEMATODE SENSE ORGANS IN UNDERSTANDING BIOLOGICAL CONTROL MECHANISMS. **Spiegel, Y.[1,2], Y. Lu[2], and S. Shaham[2].** [1]Department of Entomology and Nematology, Agricultural Research Organization, Volcani Center, P.O. Box 6, Bet Dagan 50250, Israel; [2]Laboratory of Developmental Genetics, The Rockefeller University, 1230 York Avenue, New York, New York 10065, USA.

Nematode sense organs are important in host-finding and recognition events and determining the specificity involving microbial antagonists. Therefore, they are important to understand biological control mechanisms. Amphids are the largest, most important sensory organ of nematodes. These bilateral organs consist of 12 sensory neurons and two glial cells, referred to as sheath and socket cells. At the tip of the nematode head these two come together to form a tube – the amphid channel, which contains a material, the matrix, of which some components are secreted by the sheath glia. These 12 neurons have been studied mainly by attraction assays and genetic tools. There is, however, little knowledge about the matrix composition and origin. By labeling wild-type and various mutants (cilia and glia-ablated) of the nematode *Caenorhabditis elegans* with fluorescent and gold-conjugated lectins (carbohydrate-binding proteins), we were able to gain preliminary information about the nature and localization of carbohydrates in the amphids and phasmids glia matrix. N-acetyl-glucosamine (GlucNac) residues are located, in wild type species, in different glia cells around the sensory cilia extended from the neuron dendrites within the amphid channel, and also protracted anteriorly and posteriorly to the amphid channels. Mannose residues are located mainly anteriorly and within the glia cells within the amphid channel and in the inner/outer labial and cephalic neurons in the tip of the nematode 'nose'. Phasmids reveal the presence of both carbohydrate moieties. In several mutants (e.g. *che-2* or *osm-6*), carbohydrate were presented only in the anterior part of the glia cells, and slim label observed in the phasmid. Amphid neuron dye filling defected species (*fig-1* mutant) did not exhibit neither GlucNac, nor mannose residues in both amphids and phasmids. As sensory organs are conserved structures within the phylum Nematoda, the knowledge gained by *C. elegans* could be expended to plant-parasitic nematodes as well.

THE CHEMICAL AND NEURAL BASIS OF INNATE BEHAVIORS IN *C. ELEGANS*. **Srinivasan, J., L. Aurilio, C.D. Chute, D.K. Reilly, V. Coyle, V. Nikolaki, A. Burns, and F. Ong.** Department of Biology and Biotechnology, Worcester Polytechnic Institute, Worcester, MA 01609.

Innate behaviors are mediated by the integration of external stimuli and internal signals. These cues often take the form of small biogenic molecules, ranging from exogenous secreted compounds to endogenous neuromodulators and govern behavior across all domains of life. The soil dwelling nematode *C. elegans* uses a family of small molecules called ''ascarosides'' to signal a multitude of information about the physical and social environment. Within these social cues are gender-specific behaviors, including hermaphrodite repulsion and male attraction. Although the basic components have been characterized in complex systems, the underlying physiological and neural mechanisms are largely unknown. *C. elegans* is an ideal organism for studying the relationship between small molecules and behavior due to its ability to survive in an unpredictable habitat. In order to successfully navigate their environment, these nematodes must sense and respond to multiple external cues, including physiological state-dependent signals produced and secreted by conspecifics communicating important ecological contexts. Our laboratory is interested in characterizing the chemical and neural basis of response to these complex cues. We will present evidence of two different biogenic molecules that mediate repulsion and attraction in *C. elegans*.

DEVELOPMENT OF *ROTYLENCHULUS RENIFORMIS* DIFFERS ON RESISTANT *GOSSYPIUM BARABDENSE* ACCESSIONS TX 110 AND GB 713. **Stetina, S.R.** USDA ARS Crop Genetics Research Unit, PO Box 345, Stoneville, MS 38776.

Two *Gossypium barbadense* lines (cultivar TX 110 and accession GB 713) have been used to develop *G. hirsutum* (cotton) germplasm lines with resistance to reniform nematode (*Rotylenchulus reniformis*). Their effects on reniform nematode development and fecundity were documented in three repeated growth chamber experiments, with susceptible *G. hirsutum* cultivar Deltapine 16 as a control. Nematode development on roots early (1 to 5 days after inoculation; DAI) and late (5 to 25 DAI) in the infection cycle was measured at set intervals. Genotypes were compared based on the number of nematodes in four developmental stages (vermiform, swelling, reniform, gravid). Egg production by individual females parasitizing each genotype was measured at 15, 20 and 25 DAI. Early in the infection cycle, development occurred one day faster on susceptible cotton than on the resistant genotypes. Progression to the reniform and gravid stages of development later in the infection cycle occurred first on the susceptible genotype, followed by *G. barbadense* cultivar TX 110, and finally *G. barbadense* accession GB 713. There were no significant differences in egg production by nematodes infecting the three genotypes. This study is the first report of delayed development associated with *G. barbadense* accession GB 713, and the different developmental patterns in this genotype and *G. barbadense* cultivar TX 110 suggest that unique or additional loci may confer resistance in these two lines.

AN 'OMICS' BASED APPROACH TO THE PATHOGENIC MECHANISM OF PINE WILT DISEASE. **Takeuchi, Y.[1], A. Kaneko[1], T. Kato[1], M.N. Honjo[2], A.J. Nagano[2,3], H. Kudoh[2], K. Mori[4], T. Kikuchi[5], S. Kuhara[4], and K. Futai[1].** [1]Graduate School of Agriculture, Kyoto University, Kyoto 606-8502, Japan; [2]CER, Kyoto University, Shiga, 520-2113, Japan; [3]PRESTO Researcher, JST; [4]Faculty of Agriculture, Kyushu University, Fukuoka 812-8581, Japan; [5]Faculty of Medicine, University of Miyazaki, Miyazaki 89-1692, Japan.

Pine wood nematode (PWN, *Bursaphelenchus xylophilus*) is the causal agent of pine wilt disease, which has devastated Japanese pine forests for more than 100 years. It is a mycophagous and phytophagous nematode and transmitted from tree to tree by cerambycid beetle of the genus *Monochamus* to cause disease. Since the draft PWN genome sequence was published in 2011 (Kikuchi et al., 2011), molecular basis of this complex disease has been steadily upgraded. As an example of recent 'omics' studies based on the genome information, here we introduce an integrated approach of classical genetics and genomics to the pathogenic determinant(s) of PWN.

We established a set of recombinant inbred lines (RILs) of *B. xylophilus* from two inbred lines, F7 and P9, which greatly differ in virulence. Using these lines as experimental materials, we conducted phenotyping and genotyping in order to determine the responsible genes for three important pathogenicity-related traits, namely, virulence (degree of pathogenicity), reproductive ability and boarding ability on the vector beetle. As a result, RILs showed a wide variety in virulence and reproductive ability along a continuum and two distinct boarding abilities. This indicates that virulence and reproduction may be quantitative, polygenic trait, while transmission ability is a qualitative trait which is controlled by a single or few genes. In genotyping by RAD-seq (restriction-site associated DNA sequence) analysis, PWN Ka4 isolate that is available on the GeneDB website (v1.2) was used as reference sequence, which allowed the discovery of ~25,814 SNPs (single nucleotide polymorphisms). Analysis for association between those SNPs and above-mentioned traits identified several candidates for gene loci responsible for the traits, followed by gene annotation.

In the presentation we will propose a possible pathogenic mechanism of PWN in relation to the three traits and introduce future prospects of this study.

BIOLOGICS FROM BAYER'S PERSPECTIVE. **Tarver, M.** Bayer CropScience LP, Biologics, 890 Embarcadero Drive, West Sacramento, CA 95605.

Many broad-spectrum fumigants for soil disinfection or effective chemical nematicides of the organophosphate (OP) or carbamate class are of toxicological or environmental concern, and will disappear or be severely limited in their use. Today there are rather few alternatives available to manage serious nematode pressure. A number of new chemical and biological products are developed by the crop protection industries which have better safety profiles. Thus, it is advisable to combine the use of the new products in an integrated approach, providing diversity by making use of their specific properties and strength. We will discuss examples for such integrated solution proposals based on the current and upcoming Bayer CropScience portfolio for nematode control.

DEVELOPING A PARTIAL RESISTANCE TEST FOR FODDER RADISH (*RAPHANUS SATIVUS* VAR. *OLIEFORMIS*) AGAINST *MELOIDGYNE CHITWOODI*. **Teklu, M.G.[1,2], T.H. Been[1], and C.H. Schomaker[1].** [1]Wageningen University and Research Centre, Plant Research International, Agro-systems research, NL- 6700 AA, Wageningen, The Netherlands. [2]Laboratory of Nematology, Wageningen University, 6708 PD Wageningen, The Netherlands.

Since its detection in The Netherlands in 1990, *M. chitwoodi* became a major threat to seed and ware potato production. An integrated approach to control this nematode is needed and first steps have been taken. One of these is the availability of resistant green manure crops. The EU project QLRT-1999-1462 (DREAM), proved the feasibility to produce resistant green manure crops against *M. chitwoodi* and *M. fallax*. As a result, breeders in The Netherlands and Germany introduced partially resistant fodder radish varieties.

Partial resistance in fodder radish is the result of a mixture of susceptible and resistant seeds. Current methods do not reflect the real host-status of these varieties, they provide resistance based on *P*f/*P*i ratio, which is known to be density dependent. Moreover, they are tested in an artificial system that ignores the relevant conditions set by the population dynamics of this nematode. Therefore, the development of a reliable method to estimate the partial resistance of these fodder radish varieties is of major importance.

Teklu *et al*., (2014) indicated that studying the population dynamics using a range of *P*i values and a specific experimental setup made it possible to estimate partial resistance of fodder radish. A second experiment with two new varieties (Melotop and POR1101), two previously tested ones (Defender and Contra) and two standard controls (Radical and Siletina) was carried out to ascertain the reproducibility of the first results, check the *P*i independence of the partial resistance estimator and assess the proportion of resistant seeds in the seed mixture. Ultimately, this research has to lead to a simplified screening method at one/two *P*i densities while maintaining the validity and precision of the estimator.

The biomass of all plant parts were assessed and correlated with the *P*i to investigate the magnitude of any negative effect of plant growth on the sanitary effect of this green manure.

Not all tested varieties showed tolerance to *M. chitwoodi*. Minimum yield (*m*) varied between 0 and 0.8. The number of roots with galls increased with *P*i and was highest in both controls. The results confirm the use of population dynamic models to define partial resistance. Maximum multiplication rates (*a*) and maximum population densities (*M*) were very low. The two standard varieties, Radical and Siletina, (a = 0.116 and 0.136 and M = 1.85 and 1.12 J2 (g dry soil)$^{-1}$, respectively), proved to be bad hosts. Partial resistance based on the more reliable parameter M was high ($RS_M < 1\%$) for tested varieties. RS_a and RS_M were not always equal, as estimation of a was difficult, due to the low nematode counts and higher standard errors at low *P*i values of the resistant varieties. Possibilities to use fewer nematode densities will be discussed.

STUDY OF PRE-HATCH DEVELOPMENT OF *HETERODERA GLYCINES* (SOYBEAN CYST NEMATODE) EGGS IN A MICROFLUIDIC DEVICE. **Thapa, S. and N.E. Schroeder.** Dept. of Crop Sciences, University of Illinois, Urbana-Champaign. Turner Hall, 1102 South Goodwin Avenue, Urbana, IL, 61801.

Soybean cyst nematode (SCN)-*Heterodera glycines* is a devastating pest of soybean worldwide in terms of yield loss. It is the most important pathogen of soybeans in United States and found throughout all soybean growing regions. SCN is an obligate endoparasitic pathogen. Females lay eggs in a jelly-like mass attached to their posterior end and retain about two-thirds of the eggs within their swollen bodies. Second-stage juveniles (J2) infect soybeans. Under ideal conditions the life cycle can be completed in about 21-24 days. The hatching behaviors of SCN are critical to nematode survival. These behaviors are highly coordinated to exploit the availability of suitable plant hosts and to avoid exposure to unfavorable environmental conditions. Management techniques based on hatching behaviors may be designed to control SCN. Little is known about pre-hatch development and neuromuscular mechanisms regulating hatching behaviors in SCN eggs. No information about the time taken by a fertilized SCN egg to complete the embryogenesis, J1, or J2 stages are available. Microfluidic devices are suitable substrates for such study. Microfluidic devices have recently emerged as a new technology for studies at the small scale. It has been a powerful tool for studying the free-living nematode, *Caenorhabditis elegans*. A microfluidic device was designed for SCN egg assays. Microfluidic devices were made by photolithography replica molding of a flexible silicon elastomer, polydimethylsiloxane (PDMS). The design of the device consists of a central chamber with 200 μm diameter and two ports; inlet and outlet. Each chamber holds an egg. Two- celled staged egg will be selected by crushing a cyst and will pipetted in the chamber of the PDMS replica. The hatching time of SCN eggs is different in water, host root exudate and 3 mM $ZnCl_2$ solution. These test solutions will be pipetted through the inlet port. Refilling of test solution will be done as required to keep the egg hydrated. The PDMS replicas will be kept in moisture chambers to help keeping them moist for long. Time-lapse data will be acquired using a compound microscope. The time taken to complete the embryogenesis from two-celled stage, J1, and J2 will be recorded in water, host root exudate, and $ZnCl_2$ solution. Also, the records of different cell cleavages of SCN eggs to complete embryogenesis will be made.

NEMATODE-RESISTANT COVER CROP COWPEA FOR MANAGING ROOT-KNOT NEMATODES IN SUBSEQUENTLY PLANTED VEGETABLE CROPS. **Thies, J.[1], H.F. Harrison[2], and S. Buckner[2].** [1]USDA, ARS, 605 Airways Blvd., Jackson, TN 38301; [2]USDA, ARS, Charleston, SC.

Root-knot nematode (*Meloidogyne incognita*) resistant cover crop cowpea germplasm lines (USVL-1136 and USVL-1138) developed by USDA, ARS were compared with root-knot nematode (RKN) susceptible cover crop cowpea 'Lalita' and RKN susceptible southernpea (cowpea) 'Charleston Greenpack' as cover crops for managing RKN in subsequently planted susceptible vegetable crops (romaine lettuce and okra). A clean fallow treatment and a weedy fallow treatment were also included. The studies were conducted in a *M. incognita*-infested field in Charleston, SC. In plots planted to cowpea, the resistant cowpea lines exhibited high resistance with gall indices (GI) that were lower (P <0.0001) than susceptible 'Charleston Greenpack' and 'Lalita'. Likewise, USVL 1136 and USVL 1138 had significantly lower (P <0.0001) numbers of eggs per gram fresh root than 'Charleston Greenpack' and 'Lalita'. Following the cover crop and fallow treatments, the plots were tilled and one-half of each plot was planted to okra and one-half to romaine lettuce. Differences among the four cover crop treatments, weedy fallow, and clean fallow in plots planted to okra were not detected for GI, eggs per gram fresh root, and okra fruit weight. Although differences were not statistically significant, okra grown in plots previously planted with USVL 1138 produced 97% heavier fruit than okra grown in the 'Lalita' plot treatment. Romaine lettuce grown in plots which had been planted in the USVL 1136 cover crop and clean fallow treatments had lower ($P < 0.0644$) GI than the other cowpea cover crop treatments and weedy fallow. Numbers of eggs per gram fresh root were less ($P < 0.0012$) for lettuce grown in the clean fallow compared to the other treatments. Lettuce top fresh weights were heavier ($P < 0.0637$) for lettuce grown in plots previously planted with USVL 1138 cover crop cowpea than for lettuce grown in the clean fallow and 'Lalita' cover crop cowpea treatment. Our results suggest that RKN-resistant cowpea cover crops may be useful in managing RKN in subsequently planted susceptible vegetable crops.

NATURAL SUPPRESSION OF *MELOIDOGYNE INCOGNITA* BY *PASTEURIA PENETRANS* IN COTTON. **Timper, P.[1] and C. Liu[2].** [1]USDA ARS, P.O. Box 748, Tifton, GA 31793; [2]Plant Pathology Dept., University of Georgia, 2360 Rainwater Rd, Tifton, GA 31793.

The endospore-forming bacterium *Pasteuria penetrans* is an obligate parasite of root-knot nematodes (*Meloidogyne* spp.). This bacterium is commonly found in agricultural soils and has been associated with suppression of *Meloidogyne* spp. In a field site naturally infested with both *P. penetrans* and *M. incognita*, we evaluated the effect of tillage and fumigation with 1,3-dichloropropene (1,3-D) on the abundance of *P. penetrans* spores from 2011 to 2014. We also determined whether there was a relationship between the abundance of spores and root galling in cotton. The experiment was a split-plot design with tillage (strip vs conventional) in the main plot and frequency of 1,3-D application in the subplot. There were five 4-year sequences of fumigation (C=no fumigant; F=fumigant): C-C-C-C, F-F-F-F, F-C-F-C, F-F-C-F, and C-F-F-C. Abundance of spores was determined in the spring after tillage/fumigation using a bioassay and average root-gall indices on cotton plants were determined in the fall at the time of cotton harvest. Spore abundance was greater in the C-C-C-C plots than in most of the fumigation sequences; tillage had no effect on abundance of *P. penetrans* spores. The number of spores per assay nematode was 4.9 in C-C-C-C and 2.4 averaged across fumigation treatments. There was considerable year-to-year variation in abundance of spores with a range of 11 spores/assay nematode in 2012 and 0.4 spores/assay nematode in 2014. When analyzed within a fumigation sequence, spore abundance in the spring was inversely correlated ($P < 0.0001$) with root galling the following fall. Fumigation with 1,3-D had only a minor effect on abundance of *P. penetrans* spores compared to the year-to-year fluctuation in abundance of spores.

EXPLORING THE USE OF BLACK SOLDIER FLY *HERMETIA ILLUCENS* (DIPTERA: STRATIOMYIDAE) AS AN *IN VIVO* ENTOMOPATHOGENIC NEMATODE REARING HOST. **Tourtois, J., J. Ali, and M. Grieshop.** Department of Entomology, Michigan State University, East Lansing, MI 48823.

Entomopathogenic nematodes are broadly effective and organically acceptable biological control organisms for a multitude of soil dwelling insect pests; however, they are too expensive for many small organic farmers. Using black soldier fly larvae, *Hermetia illucens* (Diptera: Stratiomyidae), as rearing hosts could make entomopathogenic nematodes more adoptable. Black soldier flies can provide multiple on-farm benefits, the largest of which is the transformation of organic wastes into compost and a nutritious livestock feed. In preliminary experiments infective juveniles emerged from only a few black soldier fly cadavers; however, upon dissecting the cadavers, thousands of dead juveniles were found inside the cadaver. The objective of this project was to develop an optimized entomopathogenic nematode rearing procedure using black soldier fly larvae. We compared the susceptibility of multiple life stages (2nd instars, 4th instars, 5th instars, and prepupae) of black soldier fly and mature wax worm larvae to four species of nematodes: *Heterorhabditis bacteriophora* (Rhabditida: Heterorhbditidae), *Steinernema carpocapsae* (Rhabditida: Steinernematidae), *S. feltiae,* and *S. riobrave*. We assessed insect mortality daily for seven days, and then cadavers were frozen and dissected to tabulate the number of nematodes that infected the host. We found that black soldier fly were not an optimal host to rear entomopathogenic nematodes due to very low infection rate. Next, we injured fifth instars by poking them 18 times with an insect pin and then exposed them to infective juveniles of the same four nematode species. We assessed insect mortality daily for five days, and then dissected the cadavers to tabulate the number of nematodes that infected the host. Injuring the black soldier fly dramatically increased mortality rate when infected with *Steinernema spp*., but not *H. bacteriophora*. Later, fly larvae were injured before infection and cadavers were injured again a week later. We assessed insect mortality and collected infective juveniles in White traps. Injuring the black soldier fly larvae before or after infection did not significantly increase the number of infective juveniles harvested compared to non-injured host. Injuring the black soldier fly larvae both before and after infection significantly increased the number of *H. bacteriophora* and *S. carpocapsae* infective juveniles harvested compared to *S. feltiae* and *S. riobrave*; however, at least 10 times more nematodes were produced per gram of *Galleria mellonella* (Lepidoptera: Pyralidae). In a follow-up experiment, infective juvenile movement towards injured and non-injured hosts was measured in an olfactometer. Injuring the black soldier fly larva did induce increased movement by *H. bacteriophora*. The major conclusion of this study was black soldier fly were not susceptible to entomopathogenic nematodes. Host modification by injury improved infection rates, but it did not sufficiently improve host quality to use this insect as a rearing host.

METAGENOMICS FOR ADVANCING NEMATODE SYSTEMATICS AND PHYLOGEOGRAPHY. **Thomas, W.K.** Hubbard Center for Genome Studies, University of New Hampshire, 35 Colovos Road, Durham, NH 03824.

Since the advent and development of the polymerase chain reaction in 1980s, molecular approaches have had an ever-increasing influence on our understanding of nematode diversity. Early application of that technology provided a phylum-wide understanding of nematode evolutionary history placing our vast knowledge of nematode biology in an evolutionary framework and linking our exceptional laboratory models (*Caenorhabdidtis* and *Pristionchus*) with the important biology of agricultural pests as well as animal and human pathogens. As technology and bioinformatics approaches advance, we have expanded our molecular understanding of nematodes with many well-developed comparative genomic models. More recently, the dramatic reduction in the cost and time required for DNA sequencing driven by the personal genomics

revolution has transformed our ability to investigate nematode biodiversity. This was first manifest as an ability to conduct molecular taxonomic analysis by sequencing phylogenetically informative genes amplified directly from environmental samples. That ability to conduct environmental sequencing has given us great insights into nematode diversity on scales that would have required ''an army of nematode taxonomists'' a few short years ago. More recently, continued improvements in sequencing cost reductions and the development of useful computational tools have made it possible to imagine moving beyond the sequencing of common phylogenetically informative loci and describing the changes in community structure associated with environmental changes to a true metagenomics approach where we sequence the ''genomes'' of environments. Such advances will allow us to consider the metabolic potential of communities and take an important step to understanding the ecological roles of nematodes and their associated organisms. Currently, the greatest limitation is not the sequencing costs as much as the availability of well annotated reference genomes with which to compare environmental sequences.

INSIGHT INTO THE SOYBEAN TRANSCRIPTIONAL PROFILING UPON INFECTION BY ROOT LESION NEMATODE. **Vieira, P.[1,2], S. Wantoch[2], J.D. Eisenback[1], and K. Kamo[2].** [1]Dept. of Plant Pathology, Physiology, and Weed Science, Virginia Tech, Blacksburg, VA 24061; [2]USDA-ARS/US National Arboretum, Floral and Nursery Plants Research Unit, 10300 Baltimore Ave., Building 010A, Beltsville, MD 20705.

Worldwide crop losses due to plant-parasitic nematodes have been estimated at $118 billion annually, with *Pratylenchus* spp. (commonly known as root-lesion nematodes, RLN) ranking third in terms of economic losses. Despite the use of management strategies such as crop rotation with non-host species, sustainable and long-lasting pest control strategies are in high demand. The range of available nematicide products is also limited, as most of these will be banned in the future. Global parasite-host transcriptomes constitute an excellent tool to provide a general overview of the molecular dialogue established between the pathogen and the host, and ultimately for the identification of the main host molecular pathways and individual genes involved during the host-pathogen interaction. Local transcriptional changes upon *Pratylenchus penetrans* infection were evaluated at two time points after nematode infection (3 and 7 days), using mRNA-seq analyses in an economically important crop, such as soybean. Preliminary data on the soybean data sets allowed the identification of a dynamic expression of genes in infected roots, suggesting a strong involvement of several metabolic pathways such as the phenylalanine and phenylpropanoid biosynthesis. The regulation of plant defense genes and reduction oxidation genes appear to play an important role during root lesion disease induction.

EFFECT OF SPIROTETRAMAT ON HATCH AND PENETRATION OF *ROTYLENCHULUS RENIFORMIS*. **Waisen, P. and B. Sipes**. Department of Plant and Environmental Protection Sciences, University of Hawaii at Manoa, Honolulu, HI 96822.

Spirotetramat is a lipid biosynthesis inhibitor effective against some insect pests and plant-parasitic nematodes such as *Meloidogyne*, *Heterodera*, *Pratylenchu*s, and *Tylenchulus*. Since it has traits and behaviors that inhibit lipid biosynthesis, it would be logical to investigate if spirotetramat would affect *Rotylenchulus reniformis*. A laboratory experiment was conducted to assess the effect of spirotetramat on nematode egg hatching. Twenty eggs of *R. reniformis* were placed in BPI dishes filled with 5 ml of solutions equivalent to 0, 50, 100, or 200 g a.i./ha. Hatched nematodes were counted at 2, 4, and 6 days later. A greenhouse experiment was conducted to determine the penetration of tomato roots by *R. reniformis*. Tomato seedlings were foliar sprayed with spirotetramat equivalent to 0, 50, 100, or 200 g a.i/ha. 14 days after inoculation with 1000 eggs of *R. reniformis*. Two weeks later, roots were collected, stained with acid fuchsin, and number of nematodes penetrating were recorded. Spirotetramat did not suppress the hatching of *R. reniformis* nor decrease nematode penetration even as the concentration of spirotetramat increased. Lack of effects from spirotetramat on egg hatch in the laboratory incubation test is probably because the compound has no contact activity and only the secondary metabolite is active against the nematodes. The metabolite may not have been formed or mobile across the nematode egg shell, thus there was no effect on hatch.

GENETIC VARIATION OF SOYBEAN CYST NEMATODE RACES AND RESISTANCE EVALUATION OF SOYBEAN VARIETIES IN NORTHERN CHINA. **Wang, C.[1], C. Hua[1], S. Chen[1], C. Li[1], Y. Mao[1], C. Zhou[2], F. Pan[1], Y. Hu[1], and Z. Tian[2].** [1]Key Laboratory of Mollisols Agroecology, Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences, Harbin 150081, China; [2]Daqing Branch, Heilongjiang Academy of Agricultural Sciences, Daqing 163000, China.

Soybean cyst nematode (SCN, *Heterodera glycines*) is the most economically important pathogen of soybean (*Glycine max*) worldwide. SCN race 3 has been the dominant race in Heilongjiang Province, the largest production province of soybean in China. Reduced or lost resistance in some soybean varieties resistant to major SCN race 3 was observed in the field, indicating change of SCN races. Yet it was not clear how SCN races were changed in the field and what is the reaction of local soybean varieties to the changed SCN. In this study, two soil samples were collected from the field of Anda District where the SCN races had changed from the initial identification. One race 4 of SCN collected from Shandong Province was used as a control. A single cyst from each soil sample was cultured and reproduced on susceptible soybean. Then 4 differential hosts and a standard susceptible cultivar Lee 68 were inoculated and numbers of cysts in the soil and on the root were quantified 35-40 days after inoculation. Eggs were

collected from these cysts. Two samples from Anda District were identified as SCN race 4 based on the race determination schemes of Riggs and Schmitt, which was same as SCN race 4 from Shandong. However, dramatic variations in the number of eggs per plant were observed between SCN race 4 from Shandong and the two samples from Anda. Yet both the field test and greenhouse test consistently showed variation of SCN races from race 3. The greenhouse test suggested the two samples might be race 4, but with virulence difference compared with race 4 from Shandong based on the number of eggs per plant. Then 12 resistant cultivars (major resistant background from Peking) and 5 varieties susceptible to SCN race 3 were inoculated with identified SCN race 4 from Shandong and Anda District. The female index and egg production index compared to susceptible Lee 68 genotype were evaluated. Among 17 varieties, the range of the female index was 58-113/plant for race 4 from Shandong and 25-109/plant for Anda race 4. The egg production index was 47-250/plant for race 4 from Shandong and 7.6-139/plant for race 4 from Anda District. The broken resistance of soybean varieties to SCN race 3 confirmed that SCN race was changed in the field and that evaluation of resistance by using egg production from cysts might be another important indicator.

CONTRIBUTION OF NO-TILL COVER CROPPING TO GREENHOUSE GAS REMEDIATION: CAN NEMATODES TELL THE TALE? **Wang, K-H.[1], G. Chen[2], Z. Cheng[1], M. Quitanilla-Tornel[1], and C.R.R. Hooks[2].** [1]Plant and Environmental Protection Sciences Department, University of Hawaii, Honolulu, HI; [2]Entomology Department, University of Maryland, College Park, MD.

One of the many benefits of no-till cover cropping is the remediation of greenhouse gas (GHG) emissions compared to conventional-tilled farming. One might argue that conventional till followed by plastic mulch would also reduce GHG emissions. This study investigated if improving soil health or keeping the soil covered play important roles in reducing GHG emissions in agroecosystems. A field trial was conducted in Upper Marlboro, Maryland in 2012 and 2013 comparing four treatments: 1) conventional tillage without surface mulch (bare ground, BG), 2) conventional tillage with black plastic (BP), 3) strip-till (ST, 30 cm wide strip) and 4) no-till (NT). Mixed winter cover crops, forage radish (*Raphanus sativus*), crimson clover (*Trifolium incarnatum*) and rye (*Secale cereal*) were planted in the fall in all plots and terminated with flail-mowing in May (at flowering). Eggplant (*Solanum esculantum*) was grown in the summer of 2012, whereas sweet corn (*Zea mays*) was grown in 2013. Soil health conditions were monitored using nematode community analysis at termination of cover cropping, one month after cash crop planting, and at crop harvest. Greenhouse gas (N_2O) emissions from the planting rows were frequently sampled after field operations, fertilizations, irrigations, and rainfall events. Mulching (BG vs BP, NT, ST) only affected abundance or % of herbivorous and bacterivorous nematodes in both years. However, conservation tillage (ST, NT) reduced abundance of bacterivorous, but increased fungivorous, omnivorous (in 2012) and predatory nematodes (in 2013) as compared to conventional till (BG, BP). In 2012, conservation till enhanced fungal decomposition (higher channel index, CI). In 2013, conservation till enhanced soil food web structure (higher maturity index, MI and structure index, SI). N_2O emissions were highest in BP, higher than that in ST and NT ($P < 0.05$) during the crop growing season in both years. Canonical correspondence analysis (CCA) between abundance of nematode trophic groups and environmental data (N_2O emissions, EI, CI, SI, MI and diversity) revealed that N_2O emissions were positively correlated to enrichment index (EI), while negatively correlated to CI, MI and SI in both years. While abundance of omnivorous nematodes was negatively related to N_2O emissions in 2012, that of herbivorous and bacterivorous nematodes were positively related to N_2O emissions in 2013. These data implied that more structured or fungal dominated soil food webs were associated with lower GHG emissions. Whereas mulching with BP resulted in the highest GHG emissions. Despite no difference in soil organic matter, active carbon, and soil aggregates stability among soil treatments within the 2 years of study, differences were detected among nematode indices within 1 year, and were corresponded to GHG emissions. Eggplant yield in NT was lower than all other treatments in 2012, but corn harvest were higher in ST than all other treatments in 2013.

CLE SIGNALING IN THE COMPATIBLE INTERACTION BETWEEN POTATO AND POTATO CYST NEMATODES. **Wang, X.[1,2], S. Chen[2], S. Zhang[3], W.S. De Jong[2], and M.G. Mitchum[4].** [1]USDA-ARS, Robert W. Holley Center for Agriculture and Health, Ithaca, NY 14853; [2]School of Integrative Plant Science, Cornell University, Ithaca, NY 14853; [3]Proteomics and Mass Spectrometry facility, Institute of Biotechnology and Life Science Technologies, Cornell University, Ithaca, NY 14853; [4]Division of Plant Sciences and Bond Life Sciences Center, University of Missouri, Columbia, MO 65211.

The potato cyst nematodes (PCN) *Globodera rostochiensis* and *G. pallida* are devastating potato pests of quarantine significance. Genes encoding effector proteins that have sequence similarity to a family of plant CLAVATA3/ESR (CLE)-like proteins have been cloned from the two PCN species as well as *G. ellingtonae*, a new *Globodera* species recently detected in Oregon and Idaho. Prior studies have demonstrated that cyst nematode-secreted CLE effectors mimic host plant endogenous CLE peptides to manipulate plant developmental pathways, thereby promoting successful nematode parasitism. Using ectopic expression coupled with nanoLC-MS/MS analysis, we have recently shown that the *in planta* functional form of GrCLE1, a multidomian CLE effector secreted by PCN during infection, is a 12-amino acid arabinosylated glycopeptide with striking structural similarity to mature plant CLE peptides. Candidate CLE receptors including *StCLV2* have been cloned from potato and confirmed to interact with PCN-secreted CLE peptides by *in vitro* receptor-peptide binding assays. Significantly, transgenic potato lines with reduced *StCLV2* expression showed enhanced

resistance to the two PCN species, suggesting that interference with nematode CLE-mediated signaling pathway may confer broad-spectrum resistance in potato against PCN pests. We are in the process of screening selected wild potato accessions to identify non-responsive *StCLV2* alleles, using an *in-vitro* CLE peptide application assay. The identification of non-responsive CLE receptor alleles may facilitate the understanding of host receptor perception of nematode CLE peptides and lead to novel methods for generating nematode resistance in potato.

INHIBITORY EFFECTS OF *GLEDITSIA SINENSIS* (CHINESE HONEYLOCUST) POWDERED FRUITS AND EXTRACTS ON *MELOIDOGYNE INCOGNITA*. **Wen, Y.[1], D.J. Chitwood[2], and S.L.F. Meyer[2].** [1]Guangdong Province Key Laboratory of Microbial Signals and Disease Control, South China Agricultural University, Guangzhou 510642, China; [2]Nematology Laboratory, USDA Agricultural Research Service, Beltsville, MD 20705.

The Chinese honeylocust (*Gleditsia sinensis*) has been used in traditional Chinese medicine, including as an anthelmintic. Consequently, mature fruits (seeds and pods) of *G. sinensis* were tested for activity against plant-parasitic nematodes. An ethanolic extract was prepared from dried, ground fruit powder: 200 g powder was soaked for 5 days in 1.6 L 95% ethanol, filtered, and collected, and the residue extracted 5 days in 1.0 L 95% ethanol, filtered, and combined with the first extract. The ethanol was removed with rotary evaporation. In microwell culture plates, eggs (ca. 100 per well) and J2 (ca. 50 per well) of *Meloidogyne incognita* were immersed in four concentrations: 1) 10 mg/ml aqueous *G. sinensis* extract; 2) 1.0 mg/ml; 3) 0.1 mg/ml; or 4) deionized water control. Five replicates were tested in each of one to three trials. Numbers of hatched and/or active vs. inactive nematodes were counted after 3 and 7 days (egg assays) or after 1, 2, 3, 4 days and a water rinse (J2 assays). *Gleditsia* powder and extract were tested in the greenhouse for activity against *M. incognita* on pepper (*Capsicum annuum*) 'PA-136' in steam-pasteurized soil. Pepper seedlings were transplanted into soil treated with: 1) 0.8% *Gleditsia* powder (dry weight:weight dry soil); 2) 1.2% w:w; 3) 1.6% w:w; 4) a drench of 60 ml water + 60 ml *Gleditsia* extract (24 ml in 960 ml water); 5) water control; or 6) water control without nematodes. Soil in treatments 1-5 was inoculated with 5,000 *M. incognita* eggs in 1 ml of water per pot. There were eight replicate pots per treatment in each of two trials (N=16). Plants were harvested 8 weeks after transplant, plant vigor measured, and eggs extracted from roots. In the laboratory assays with J2 in extracts, all three treatments resulted in decreased J2 viability. Egg hatch was reduced in the 10.0 and 1.0 mg/ml concentrations. In the greenhouse, transplanting into pots treated with powder or extract resulted in decreased shoot lengths and shoot and root weights compared with water controls. All *Gleditsia* powder amendments resulted in lower numbers of galls on the pepper roots. However, 0.8% and 1.2% *Gleditsia* powder each increased eggs/g root in one trial, while 1.6% *Gleditsia* powder decreased number of eggs/g root in one of two trials. *Gleditsia* fruit demonstrated nematicidal activity, but high rates may be needed for suppression of *M. incognita* in the soil, and a waiting period might be indicated between application of powdered amendment and transplant into the treated soil.

EFFECT OF APPLICATION TIMING AND IRRIGATION ON MANAGING NEMATODES ON CARROTS WITH NIMITZ 15G. **Westerdahl, B.B.** Department of Entomology and Nematology, University of California, Davis CA.

Two RCB field trials with 5 replicates per treatment were conducted to evaluate the effectiveness, compared to an untreated control (UC), of Nimitz 15G (fluensulfone, MCW-2, ADAMA, NC) for management of root-knot nematode (RKN), *Meloidogyne javanica*, on carrot. Treatments in the first trial, were UC, 1,3-Dicholorpropene (Telone II, 1,3-D, Dow AgroSciences, Indianapolis, IN), Nimitz 15G at 13.1, 18.7, and 26.2 kg/ha applied 20 days pre-plant followed by mechanical incorporation and a single irrigation; and the same three Nimitz 15G treatments followed by irrigation at 0, 5, 10, and 15 days following mechanical incorporation. Treatments in the second trial were UC, 1,3-D, and the same three rates of Nimitz 15G as in the first trial, applied 20, 30, or 45 days pre-plant followed by incorporation and a single irrigation. In the first trial, all multiple irrigation treatments had a greater total number of marketable carrots than UC ($P = 0.05$). The multiple irrigation treatments at 13.1 and 18.7 kg/ha also had a greater total weight of marketable carrots than UC ($P = 0.05$). The 18.7 and 26.2 kg/ha single irrigation treatments had fewer RKN at harvest than UC ($P = 0.05$). In the second trial, 1,3-D had a greater total number and weight of marketable carrots than untreated ($P = 0.05$). There was a trend for longer pre-plant intervals and higher rates of Nimitz 15G to have a greater total weight and total number of marketable carrots than UC. At harvest, the 18.7 kg/ha treatment at 45 days pre-plant, and 1,3-D had fewer RKN in soil than UC ($P = 0.05$). There was a trend for the 45-day pre-plant treatments to have fewer RKN than did the 30 and 20-day treatments.

EFFECT OF CROP ROTATION, IRRIGATION RATE, AND CULTIVAR ON COTTON YIELD AND VALUE IN A FIELD INFESTED WITH *MELOIDOGYNE INCOGNITA*. **Wheeler, T.A. and J.W. Keeling.** Texas A&M AgriLife Research, Lubbock, TX 79403.

The effect of cotton rotation with sorghum was tested from 2008 to 2010, with one year of sorghum followed by two years cotton (SCC); or from 2012 to 2014 with one year wheat/fallow alternated with one year cotton (WC). Both rotation systems were compared against continuous cotton (CCC). All cropping systems included three irrigation rates (1.0B = base rate; 1.3B = 30% above 1.0B; 0.7B = 30% below 1.0B); and partially resistant and susceptible cotton cultivars. *Meloidogyne incognita* was considered a moderate stress factor for cotton on the susceptible cultivar during the sorghum rotation study (fall density

averaged 5,416/500 cm^3 soil). *M. incognita* was a low stress factor for the wheat/cotton study during 2012 and 2013 (fall density averaged 562/500 cm^3 soil). The low density was a function of the severe drought conditions experience in 2011and 2012. *M. incognita* was a low to moderate stress factor on the susceptible cultivar in 2014 (fall density =2,858/500 cm^3). Cotton lint yield following sorghum which is a host of *M. incognita*, was 13% higher than CCC (1328 kg/ha vs 1173 kg/ha, respectively). Cotton following a winter wheat/fallow rotation yielded 41% higher than CCC (1099 kg/ha vs 777 kg/ha, respectively). A partially resistant cultivar to *M. incognita* yielded 33% higher than a susceptible cultivar during the sorghum/cotton study and 10% higher during the wheat/cotton study. The SCC rotation returned $74/ha more than CCC when cotton lint was priced at loan value or $50/ha less than CCC when cotton lint price was approximately $1.54/kg lint (adjusted by $1.15/kg lint – actual loan value to account for fiber quality differences). The WC rotation returned $369/ha more than CCC at loan value and $284/ha more than CCC when lint was approximately $1.54/kg. Yield and gross margins increased with increasing irrigation rate for both rotation studies. Cotton lint averaged 1026, 1275, and 1450 kg/ha at 0.7B, 1.0B, and 1.3B, respectively in the sorghum/cotton trials; and 734, 977, and 1105 kg/ha, respectively in the wheat/cotton trials. At lint values of approximately $1.54/kg, the gross margin for 0.7B, 1.0B, and 1.3B in the sorghum/cotton trials were $88, $300, and $397/ha, respectively. The gross margin for 0.7B, 1.0B, and 1.3B in the wheat/cotton trials were $−329, $−121, and $−83/ha respectively. Crop rotation with sorghum resulted in higher yields for cotton following sorghum and marginally improved gross margin if cotton prices were low. However, the value of SCC was negated as cotton prices increased. The wheat/fallow rotation improved cotton yield and gross margin substantially compared with continuous cotton in the range of cotton prices analyzed. Winter wheat is unlikely to increase *M. incognita* density because of temperature and soil moisture constraints and none were recovered during fall sampling after the summer fallow. Nematode densities rebounded following the cotton crop.

GEOGRAPHIC DISTRIBUTION AND GENETIC DIVERSITY OF *BAKERNEMA INAEQUALE*. **Whitlock, K.[1], E.C. Bernard[1], and T.O. Powers[2].** [1]Entomology & Plant Pathology, University of Tennessee, 2505 E. J. Chapman Drive, 370 Plant Biotechnology, Knoxville, TN 37996-4560; [2]Department of Plant Pathology, University of Nebraska-Lincoln, Lincoln, NE 68583-0722.

Plant-parasitic nematodes of the family Criconematidae (ring nematodes) are distributed worldwide. Although several species are significant pathogens of turf and fruit crops, they reach their greatest diversity in natural systems. Ring nematodes are an important component of various soil habitats, and several criconematid species often are present in a soil sample. One of the most distinctive ring nematodes is the heavily fringed *Bakernema inaequale*, endemic to eastern North America. This nematode has been found as far north as Ontario, Canada, and as far south as Tuskegee National Forest in southeast Alabama. This research focused on determining the geographic boundaries and host associations of *B. inaequale*, as well as analyzing molecular data to detect any genetic variability among populations. Soil samples were collected from diverse habitats of non-agricultural land east of the Mississippi River, from the Gulf coast to New England, focusing primarily on both sides of the Appalachian chain. For this project, 203 soil samples were collected from 15 states. At each collection site, photographs were taken, GPS coordinates and elevation noted, and surrounding vegetation and general soil type documented. Soil samples remained refrigerated until processing; 150 samples were processed at The University of Tennessee, Knoxville, and the remaining 53 samples were processed at The University of Nebraska, Lincoln. After extraction, at least five living specimens from a sample were viewed individually on a differential interference (DIC) microscope, given an identification number unique to the nematode, imaged, measured, and ruptured with a micropipette tip to release DNA. Each nematode was then frozen in a PCR tube, and samples were shipped to UNL for DNA amplification using a 721 base-pair region of the cytochrome oxidase subunit 1 (COI) gene sequence. *Bakernema inaequale* was collected from 29 soil samples from seven states (Tennessee, Alabama, Pennsylvania, West Virginia, Ohio, New Hampshire, and Vermont). Prior to this study, *B. inaequale* had also been recorded from Wisconsin, New Jersey, Connecticut, Virginia, North Carolina, and Ontario, Canada. Molecular analysis indicated relatively low levels of genetic diversity among the majority of *B. inaequale*; however, specimens collected in Tuskegee National Forest (Alabama) and Accotink Creek (Virginia) differed significantly from the remaining specimen clades. Some morphological differences were noted, primarily relating to annule scale size and shape, head region, and tail. However, morphological differences were not correlated with molecular differences or geographical location. Previous documented plant host associations with *B. inaequale* include sweet gum (*Liquidambar styraciflua*), rhododendron (*Rhododendron* spp.), tulip poplar (*Liriodendron tulipifera*), American beech (*Fagus grandifolia*), Eastern hemlock (*Tsuga canadensis*), and various pine and maple species. This research also added sycamore (*Platanus* spp.), sassafras (*Sassafras albidum*), Southern magnolia (*Magnolia grandiflora*), and various oak species as likely host plants for *B. inaequale*.

ROOT-KNOT NEMATODE BEHAVIOR IN RESPONSE TO PLANT AND NEMATODE SEMIOCHEMICALS. **Williamson, V.M.[1], W.B. Danquah[1], and F. Schroeder[2].** [1]Dept. of Plant Pathology, University of California, Davis, CA 95616; [2]Boyce Thompson Institute, Ithaca, N.Y.

Root-knot nematodes (*Meloidogyne* spp.; RKN) are sedentary endoparasites that infect many crops and cause substantial losses worldwide. Infective second stage juveniles (J2) hatch from eggs in the soil and must locate and invade host roots to complete their life cycle. What attracts nematodes to roots has remained a mystery for decades. In addition, the chemical signals involved in inter-nematode communication are almost entirely unknown for plant parasitic nematodes. We have developed assays to assess RKN attraction and behavior using a thermo-reversible gel of Pluronic F-127 (PF127). These

assays are being used to identify the chemical signals that these parasites utilize to locate appropriate infection sites in the roots and to determine the roles of pheromones in this process. PF127 is nontoxic and highly transparent, and stable chemical gradients can be formed in the gel facilitating determination of the response of RKN juveniles to signals from host plants and each other. Observations in PF127 gel reveal that juveniles of root-knot nematodes (*Meloidogyne incognita*, *M. javanica* and *M. hapla*) are most strongly attracted to a region behind root tip of healthy seedlings corresponding to the zone of elongation. Cell-free tomato root exudate is attractive to nematodes, and activity-guided fractionation assays intended to identify host semiochemicals are in progress. Several behaviors indicate that RKNs communicate chemically with each other. For example, males are strongly attracted to females and juveniles aggregate into tight clumps in PF-127 gel. Ascarosides, a family of compounds that are glycosides of the sugar ascarylose with a fatty acid-derived side chain, regulate a range of social behaviors and developmental pathways in a range of nematode species. Chemical analysis of ''RKN exudate'' from juveniles reveals that ascarosides are present and that ascaroside#18 (ascr#18) is the most abundant. Incubating J2 in RKN exudate or synthetically-produced ascr#18 (10 nM) prior to exposure to tomato seedlings resulted in a significantly increased rate of accumulation at root tips compared to control treatments. J2 pre-soaked in concentrated RKN exudate aggregate into clumps overnight indicating that compounds in the RKN exudate are responsible for the aggregation. Identification of the semiochemicals that attract RKN to roots and that modify their behavior has the potential to provide tools for novel and safe strategies of control.

DEVELOPING REAL-TIME PCR ASSAYS FOR DIRECT DETECTION AND QUANTIFICATION OF ROOT-LESION NEMATODES FROM SOIL. **Yan, G.** North Dakota State University, Department of Plant Pathology, Fargo, ND 58108.

Root-lesion nematodes, *Pratylenchus neglectus* and *P. thornei*, are important nematodes that attack plant roots and restrict productivity of wheat in the Pacific Northwest (PNW). These nematodes have the potential for causing economic damage to as much as 60% of dryland wheat fields in the PNW. Pre-plant populations of these two species are frequently inversely correlated with wheat yield. It is estimated that these nematodes reduce profitability of farms in Idaho, Oregon, and Washington by about $51 million annually. The best approach to controlling damage from the lesion nematodes is to select and grow cultivars that are both resistant and tolerant. However, individual wheat cultivars differ in their reaction to each nematode species; cultivars with resistance or tolerance to one species are not necessarily resistant or tolerant to another species. Optimal cultivar selection requires that the species of lesion nematodes present in each field or region be accurately identified and quantified. It is challenging to discriminate *P. neglectus*, *P. thornei* and other closely related species based on morphological characteristics. It is difficult to use microscopy to count and identify these species in large numbers of field soil samples in which many other nematode species are also present. Quantitative real-time polymerase chain reaction (qPCR) assays were developed to detect and quantify these two species from DNA extracts of soil. The primers, designed from internal transcribed spacer region of rDNA, were highly specific to the target species and did not amplify DNA from isolates of non-target species including *Pratylenchus* spp., other nematodes, and six fungal species commonly present in PNW wheat fields. The assays were sensitive and capable of detecting genomic DNA of a single juvenile inoculated into one gram of soil. No significant difference was observed between the cycle threshold values of a single adult female, juvenile, and egg (containing faint outline of developing juvenile). Mixtures of nematodes in these life stages were used to generate standard curves by amplifying DNA extracted from soil to which nematodes were added. The standard curves were validated using sterilized soil inoculated with known numbers of *P. neglectus* and *P. thornei*. Significant positive relationships were observed for nematode numbers quantified from natural field soils using qPCR and a traditional nematode extraction method but the qPCR generally tended to provide higher estimates. Key factors (PCR inhibitor and soil moisture) were evaluated for their effects on molecular quantification of *P. neglectus* from soil. Our data indicated that reproducibility and reliability of qPCR were improved by using BSA (bovine serum albumin) and oven-dried soils. Multiple DNA extractions from each soil sample will also reduce variation caused by the small sample size and the heterogenous distribution of nematodes in soil to further improve the accuracy of the assays. Real-time PCR potentially provides a useful platform for efficient detection and quantification of *P. thornei* and *P. neglectus* directly from field soils.

STUBBY ROOT NEMATODE AS THE VIRUS VECTOR OF CORKY RINGSPOT DISEASE OF POTATO. **Yan, G. and N.C. Gudmestad.** North Dakota State University, Department of Plant Pathology, Fargo, ND 58108.

Stubby root nematodes (*Paratrichodorus* and *Trichodorus*) are of increasing importance to the U.S. potato industry due mainly to their ability to transmit *Tobacco rattle virus* (TRV), the causal agent of corky ringspot disease. TRV has become more widespread in commercial potato production areas and can cause significant internal necrosis on tubers of susceptible cultivars. The symptoms have a direct economic impact due to rejection of potatoes grown for processing or fresh market. Several stubby root nematode species are known to vector TRV including *Paratrichodorus allius,* the most prevalent vector, and *P. teres,* an important vector in the Pacific Northwest. Seven species were reported to be present in Michigan including *Trichodorus proximus*, *T. primitives, T. similis, P. christiei, P. atlanticus, P. prosus,* and *P. pachydermus*. Stubby root nematodes have also been associated with TRV-infected potato fields in California, Florida, Minnesota, and Wyoming. The severity of the disease varies depending on population densities of the nematodes present in the soil; however, a low

nematode population can still result in high incidence of the disease. Therefore, it is important to determine the population levels of the nematodes in soil to assess disease risk and enhance management strategies. Currently, nematode population densities are determined by soil extraction procedures followed by identification and counting using microscopy, a complex and time-consuming task that requires considerable nematology expertise. Sensitive, but simpler, quantitative assays would allow growers to rapidly assess fields to determine the risk of TRV prior to planting. With funds from a Specialty Crop Research Initiative (SCRI), National Institute for Food and Agriculture (NIFA) grant, we are developing real-time PCR procedures that will efficiently identify and quantify stubby root nematode populations present in the soil and predict the risk of TRV incidence to potatoes planted in that soil. Knowledge of the relationship between nematode species and population density, and TRV disease incidence and severity will be instrumental in the development and implementation of sustainable TRV management strategies.

THE STATUS OF SOYBEAN CYST NEMATODE OCCURRENCE AND MANAGEMENT IN NORTH DAKOTA. **Yan, G.[1], S. Markell[1], B.J. Nelson[1], T.C. Helms[2], and J.M. Osorno[2].** [1]North Dakota State University, Department of Plant Pathology, Fargo, ND 58108; [2]NDSU, Department of Plant Sciences, Fargo, ND 58108.

The soybean cyst nematode (SCN), *Heterodera glycines*, is a major yield-limiting factor in soybean production. Since it was first reported in North Dakota in 2003 in Richland County, infestations of SCN have spread to at least 19 soybean-producing counties as of 2014. The occurrence and distribution of SCN have been monitored through soil sampling conducted by growers and free soil testing sponsored by the North Dakota Soybean Council. SCN was detected (≥100 eggs and second-stage juveniles/100 cm3 soil) in six more counties (Benson, Cavalier, Foster, McIntosh, Stutsman, Walsh) in 2014 compared to the infested counties that have been reported previously, indicating that SCN is quickly spreading to other counties. Of 579 soil samples submitted by growers for SCN assay in 2014, 31% of the samples were found to have SCN, with a mean of 3,501 and a range of 50 to 58,500 eggs and second-stage juveniles/100 cm3 soil. Seven Plant Introduction lines with various forms of resistance and a susceptible check have been used to determine HG types of SCN in 18 infested fields from five counties (Cass, Dickey, Lamoure, Richland, Traill) using the most current method of characterizing virulence types. At the present time, only HG type 0 has been identified and confirmed in the fields tested. SCN also poses a potential threat for the dry edible bean (*Phaseolus vulgaris* L.) production in North Dakota which is the largest dry bean production area in the United States. The nematode was demonstrated to reproduce on dry bean cultivars from four classes (pinto, navy, black, kidney). SCN caused the reduction in plant growth and seed yield on three bean classes (pinto, kidney, navy) tested under field conditions. Resistance sources from plant introductions of *P. vulgaris* have been identified and SCN resistance is currently being introduced into breeding materials for the NDSU dry bean breeding program. In addition, the genetic basis for SCN resistance in dry bean is being characterized. Several nematicide seed treatment products have been evaluated in multiple locations for efficacy in suppressing SCN populations in infested fields and increasing soybean yields. However, the performance of nematicides was contingent on specific weather and soil conditions. Yield increases were observed at some locations but not always with high egg levels. Resistant cultivars combined with crop rotation are the primary methods for controlling SCN in North Dakota. Approximately 40 common soybean cultivars are evaluated for SCN resistance each year under controlled greenhouse conditions and on naturally infested SCN fields in three to four locations. The information for resistance to SCN is included in an annual bulletin of soybean performance testing and made available to growers. Corn and wheat are the most common rotational crops used by growers to reduce the population levels of SCN in highly infested fields.

USING PCR TO DETECT AND DISTINGUISH THE CEREAL CYST NEMATODES *HETERODERA FILIPJEVI* AND *H. AVENAE*. **Yan, G.[1] and R.W. Smiley[2].** [1]North Dakota State University, Department of Plant Pathology, P.O. Box 6050, Fargo, ND 58108, [2]Oregon State University, Columbia Basin Agricultural Research Center, P.O. Box 370, Pendleton, OR 97801.

Cereal cyst nematodes (CCN) are economically important nematodes that restrict wheat production in the Pacific Northwest. *Heterodera avenae* was first detected in the USA in 1974 and is now known to occur in at least seven western states. *H. filipjevi* was first detected in the USA in 2008 and is now known to occur in both Oregon and Washington. The extent to which these species occur within individual fields and regions of the western USA remains unclear. It is difficult to distinguish these species in a timely manner when using only morphometric characteristics to evaluate cysts and/or juveniles extracted from multiple fields. We therefore developed species-specific PCR and optimized PCR-RFLP to quickly and precisely distinguish these species or to provide suggestive evidence of the occurrence of other species or genera. The discovery of *H. filipjevi* in Oregon and Washington would have been unlikely without the application of molecular procedures. In both instances, the cyst nematodes occurring on wheat in certain fields of both states had been identified as *H. avenae* two or more years before they were found by PCR testing to be *H. filipjevi*. Yet it is clear that most infested fields are infested by *H. avenae*. In a recent example, during 2014, we received a sample of '*H. avenae*' from a wheat cultivar screening trial in Washington. The sample consisted of soil containing brown cysts and wheat stubble with white females embedded in roots. Initial PCR evaluations indicated that the population from the new sample was *H. filipjevi* rather than *H. avenae*. Soil samples were then collected from the initial field site and from other nearby sites. *Heterodera* spp. were detected in 16 of 36

samples. Species-specific PCR was used to identify the species. Infested fields were shown to be infested with either *H. filipjevi*, *H. avenae*, or with both species. The species identity was confirmed by comparing the PCR-RFLP restriction pattern with those of known species, sequencing the ITS region of rDNA, and examining key morphological features of the cysts. This sequence of identifications is similar to experiences in other countries where CCN species in addition to *H. avenae* have been identified. The standard PCR procedure developed in our laboratory is now offered as a service by at least one commercial nematode diagnostic laboratory that previously identified these species only to the 'cereal *Heterodera* species' group, as compared to the 'beet cyst nematode' or some other groups. Greater adoption of PCR procedures in commercial and public nematology laboratories are very likely to extend the known distribution of both *H. avenae* and *H. filipjevi* in the USA, and possibly also to detect the occurrence of the third-most important member of the '*H. avenae* group' (*H. latipons*) somewhere in North America.

DIRECT APPLICATION OF ENERGY TO SOIL FOR THE MANAGEMENT OF SOILBORNE PATHOGENS AND PLANT-PARASITIC NEMATODES. **Zasada, I.[1], J.E. Weiland[1], and L. Global[2].** [1]USDA ARS, 3420 NW Orchard Ave., Corvallis, OR 97330; [2]Richland, WA 99352.

Alternatives methods to soil fumigation are needed, especially those that minimize buffer sizes and re-entry periods. One such alternative may be the pre-plant treatment of soil with electrical current, developed by Lisi Global in Richland, WA. This method involves delivering highly concentrated electrical pulses directly into the soil. These pulses are tailored to affect specific structures in the target organism and damage or disrupt the normal function of those structures. The amount of electricity can be controlled in a way analogous to rate of application, dose, and concentration used for common management practices; in this case, joules/cc of soil. To test this concept, the plant-parasitic nematodes *Meloidogyne hapla* and *Globodera ellingtonae* and the soilborne pathogens *Phytophthora cinnamomi* and *Verticillium dahliae* were exposed to 10 to 70 joules/cc of electrical current applied to infested soil. Current is applied to the soil using the patent pending DES system developed by Lisi Global. After treatment, nematode survival was measured using hatching assays for *G. ellingtonae*, and infection assays with tomato for both plant-parasitic nematodes. Soilborne pathogen survival was measured by dilution plating on semi-selective media specific to each pathogen. Hatch of *G. ellingtonae* eggs from cysts was reduced by 83% when exposed to high current at 70 joules/cc, compared to the untreated control; hatch of *G. ellingtonae* exposed to low and medium current (20 and 30 joules/cc) was similar to that in the untreated control. On average *P. cinnamomi* densities in soil, measured as recoverable propagules per gram soil, were reduced by 55 to 67% when exposed to currents ranging from 10 to 50 joules/cc. The DES system shows promise as a pre-plant management method to reduce population densities of plant-parasitic nematodes and soilborne pathogens in soil.

CHARACTERISATION OF, AND ENTOMOPATHOGENIC STUDIES ON, *PRISTIONCHUS AERIVORUS* (RHABDITIDA: DIPLOGASTRIDAE) FROM NORTH CAROLINA, USA). **Ye, W.[1], Q. Yu[2], N. Kanzaki[3], P.R. Adams[4], and Y.J. Cardoza[4].** [1]Nematode Assay Section, Agronomic Division, North Carolina Department of Agriculture & Consumer Services, Raleigh, NC 27607; [2]Agriculture and Agri-Food Canada, Environmental Health Program / Invertebrate Biodiversity, Ottawa, Ontario K1A 0C6, Canada; [3]Forest Pathology Laboratory, Forestry and Forest Products Research Institute, 1 Matsunosato, Tsukuba, Ibaraki 305-8687 Japan; [4]Department of Entomology, North Carolina State University, Campus Box 7613, Raleigh, NC 27695-7613, USA.

During a survey of entomopathogenic nematodes in North Carolina, USA, a *Pristionchus* species was recovered using the *Galleria*-bait method. Morphological studies with light microscopy and scanning electron microscopy, mating tests with reference strains, as well as molecular analyses of the near-full-length small subunit rRNA gene (18S) and D2-D3 expansion segments of the large subunit rRNA gene (28S) identified this isolate as *Pristionchus aerivorus*. Exposed *Galleria* larvae were killed within 48 h and high numbers of nematodes were recovered from the cadavers about 5 days later. Preliminary tests revealed that this nematode is capable of infecting at least two other insect species (*Helicoverpa zea* and *Tenebrio molitor*) under laboratory conditions. The status of the genus *Chroniodiplogaster* is discussed and confirmed as a junior synonym of *Pristionchus* based on morphological observation and molecular phylogenetic analysis.

HIDING PLACES FOR *PRATYLENCHUS PENETRANS* IN RASPBERRY. **Zasada, I.[1], T.W. Walters[2], and D.R. Kroese[3].** [1]USDA ARS, 3420 NW Orchard Ave., Corvallis, OR 97330; [2]Walters Ag Research, Anacortes, WA 98221; [3]Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331.

Flat, nontarped soil fumigation with 1,3-dichloropropene and chloropicrin is the standard method for managing *Pratylenchus penetrans* in Washington raspberry fields. However, this strategy is not always effective, with population densities of *P. penetrans* returning to very high levels in some fields in as little as six months after planting. The goal of this research was to determine where *P. penetrans* is hiding in this raspberry production system. Three separate studies were conducted. First, roots containing *P. penetrans* were collected, placed in bags, and then buried at two locations and *P. penetrans* population dynamics were monitored every other month for over a year. In this study, after 10 months there were no *P. penetrans* found in residual roots at either location. In a second study, soil cores to a depth of 1 m were collected from two fields prior to

fumigation, 2 weeks after fumigation, and at planting (6 months after fumigation). *Pratylenchus penetrans* were present in the soil at all sampling dates for both fields. Post-fumigation populations tended to be concentrated at deeper depths in the field with a fine sandy loam soil with an average of 0 *P. penetrans*/100 g soil at depths of 0-50 cm and 5 *P. penetrans*/100 g soil at depths of 51-100 cm, whereas populations tended to be concentrated at shallower depths in the field with a sandy loam soil with an average of 20 *P. penetrans*/100 g soil at depths of 0-50 cm and 4 *P. penetrans*/100 g soil at depths of 51-100 cm. Finally, a common practice in raspberry production fields is to plant a wheat cover crop immediately prior to or after fumigation. To determine if wheat serves as a winter host for *P. penetrans,* wheat roots were collected several times post-fumigation and population levels of *P. penetrans* determined. Wheat was an excellent host for *P. penetrans*, with an average of 3,993 *P. penetrans*/g root found in areas of a field which had received fumigation. Combined, these data indicate that wheat may serve as a bridge for *P. penetrans* over the winter and prior to replanting of a raspberry field, that a year rotation will be necessary to exhaust *P. penetrans* populations in residual roots, and that *P. penetrans* may be escaping the effects of fumigation due to distribution throughout the soil profile down to a depth of 1 m.

THE *MELOIDOGYNE INCOGNITA* EFFECTOR MI7H08 INTERACTS WITH A PLANT TRANSCRIPTION FACTOR AND ALTERS EXPRESSION OF CELL CYCLE CONTROL GENES IN PLANT CELLS. **Zhang, L.[1,2], E.L. Davis[2], 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 27695.

The root-knot nematode (RKN) *Meloidogyne incognita* synthesizes and secretes effector proteins into the root cells of host plants to facilitate the formation of nematode feeding sites, which are comprised of several multinucleate giant cells (GCs), by manipulating various plant processes, including the cell cycle. The *M. incognita* effector Mi7H08 is the first reported nuclear-localized plant-parasitic nematode effector with transcriptional activation activity. Our current research focuses on functional characterization of Mi7H08 in RKN parasitism. Constitutive expression of *Mi7H08* in *Arabidopsis thaliana* resulted in hypersusceptibility to *M. incognita* infection, as well as longer roots and larger leaves. *Arabidopsis*-mediated RNA interference (RNAi) targeting *Mi7H08* led to a significant reduction in the number of *M. incognita* egg masses by up to 61% ($P < 0.05$) in *Arabidopsis* RNAi transgenic lines compared to empty vector controls. The results of nematode infection assays indicated a critical role of Mi7H08 for the successful nematode parasitism of plants. Mi7H08 was found to specifically interact with a plant transcription factor, which functions as a core regulator of the cell cycle. Further studies revealed that Mi7H08 is able to bind to the promoter regions of several plant cell cycle genes and activate their expression in plant cells. In summary, the transcription activator-like effector Mi7H08 plays a critical role in RKN infection, probably by directly reprogramming expression of plant cell cycle genes to facilitate the formation and maintenance of giant cells.

THE EFFECTS OF ENDOPHYTIC *PURPUREOCILLIUM LILACINUM* AND *CHAETOMIUM GLOBOSUM* ON ROOT-KNOT NEMATODE *MELOIDOGYNE INCOGNITA* UNDER GREENHOUSE AND FIELD CONDITIONS. **Zhou, W.[1], T.A. Wheeler[2], J.L. Starr[3], and G.A. Sword[1].** [1]Department of Entomology, Texas A&M University, College Station, TX 77843; [2]Texas A&M AgriLife Research & Extension Center, Lubbock, TX 79403; [3]Department of Plant Pathology & Microbiology, Texas A&M University, College Station, TX 77843.

Purpureocillium (previously *Paecilomyces*) and *Chaetomium* are reported to have negative effects against insect or nematode herbivores. *Purpureocillium lilacinum* has been formulated and commercialized as a bionematicide and bio-insecticide. We evaluated two endophytic fungal strains of *P. lilacinum* and *C. globosum*, which were isolated from cotton plants in Texas, for their endophytic effects in cotton against root-knot nematodes, *Meloidogyne incognita*, under greenhouse and field conditions. In the greenhouse experiments, introducing either of the fungi into cotton on the seed at planting suppressed *M. incognita* reproduction with reductions in egg production of 50 – 65% and 79 – 89% six weeks after nematode inoculation for *C. globosum*, and *P. lilacinum*, respectively. *M. incognita* second-stage juvenile infections at 12 days after egg inoculation were suppressed when seeds were inoculated with *P. lilacinum* (reduced on average by 60 – 75%) and *C. globosum* (reduced on average by 88 – 93%). In field trials in 2014, two seed treatment methods for the inoculation of two cotton varieties, PHY499WRF and PHY367WRF, by *P. lilacinum* and *C. globosum* were tested at two different sites. At the AGCARES site (Lamesa, TX), endophyte treatments had greatly enhanced plant performance. Plant height in PHY499WRF was increased on average by 8.4 – 10.6% in treated plots, whereas significantly more stunted plants were observed in untreated plots due to water shortage in August. Yield was significantly increased on average by 6.4% when PHY499WRF plants were treated with *P. lilacinum* using methylcellulose stickers. Within the same treatment plots, *M. incognita* eggs and juveniles were reduced on average by 63% and 67% respectively. Overall, the effects on reducing nematode populations varied depending on plant variety, endophyte, and seed treatment method.