## ABSTRACTS FROM THE SOCIETY OF NEMATOLOGISTS ANNUAL MEETING 2019

TOWARDS OPTIMIZATION OF ENTOMOPATHOGENIC NEMATODES FOR MORE SERVICE IN THE BIOLOGICAL CONTROL OF INSECT PESTS. **Abd-Elgawad**, **Mahfouz**. Plant Pathology Department, Agricultural and Biological Research Division, Elbehooth St., National Research Centre, Dokki 12622, Giza, Egypt.

Currently, entomopathogenic nematode (EPN) efficacy and cost are crucial to meeting producer and end-user needs. Despite interests in basic pillars; such as developing EPN mass culture and formulation, stability of field performance, and expectations of rapid performance; to satisfy such needs, there are a few other issues that should be addressed. Being almost impossible for nematologists to act united, they need to use standardized procedures which allows future reviews to be analytical and build on what has been done by others rather than merely descriptive. Examples of nonstandard sampling procedures of EPN that may lead to erratic results are given. Standardized sampling to grasp the interaction between EPN distribution and agricultural soil management may develop more swiftly field application techniques and/or modify the patchy distribution of EPN populations to a more uniform distribution designed to enhance EPN efficacy. Also, functional sampling should be expanded in parallel to large-scale surveys for coordination in search of new effective species/strains. Furthermore, EPN should be included in integrated pest management programs in ways that incorporate them into current management systems to make them complimentary or superior to chemical pesticides. Awareness-raising of more growers, small companies, cooperatives, and extensions of EPN as bio-insecticides should be attempted in earnest. We should better communicate the positive trends about insecticidal nematodes and demonstrate them to control pests effectively and economically.

ESTIMATE OF BROAD BEAN YIELD LOSS IN NATURALLY *MELOIDOGYNE ARENARIA*-INFESTED FIELDS. Abd-Elgawad, Mahfouz, M. M. A. Hammam, and M. M. Mohamed. Plant Pathology Department, Agricultural and Biological Research Division, Elbehooth St., National Research Centre, Dokki 12622, Giza, Egypt.

Faba bean is one of the most important grain legume crops in Egypt where its high value nutritional seeds are commonly used as food for human consumption and as feed for raising farm animals. Adequate solutions to many bean problems have been found, but more research efforts are needed to obtain more crop yield especially from recently released cultivars. Giza 843 is a new and common bean variety. It is resistant to broomrape, tolerates leaf diseases and has early flowering and relatively high yield. Yet, breeding for nematode resistance or tolerance was not included in its development program. In the present study, soil and root samples were obtained at the end of vegetative period to estimate loss in 'Giza 843' yield in *Meloidogyne arenaria*-naturally infected fields during two seasons. The relationship between numbers of nematode eggmasses, galls, or second stage juveniles in soils and green pod yield was described. The gain thresholds were 0.780 and 0.570 metric tons of pods feddan<sup>-1</sup> (= $4200 \text{ m}^{-2}$ ) for oxamyl and Nemaless, respectively, based on the combined costs of nematode sampling and control. The economic thresholds were mostly one eggmass or gall per root system or one second-stage juvenile ( $150 \text{ cm}^3 \text{ soil}$ )<sup>-1</sup> for oxamyl and Nemaless. The use of phytosanitary measures such as resistant/tolerant cultivars, crop rotation which include resistant crop(s), deep plowing, solarization, and nematicidal applications are discussed.

ENHANCING BIOLOGICAL INSECT CONTROL: PROTECTING ENTOMOPATHOGENIC NEMATODES AGAINST UV RADIA-TION AND DEHYDRATION. Acar, Ismet.<sup>1</sup> and B. Sipes<sup>2</sup>. <sup>1</sup>Plant Protection Central Research Institute, Ankara, Turkey, <sup>2</sup>Department of Plant and Environmental Protection Sciences, University of Hawaii at Manoa, Honolulu, HI, USA.

The goal of the research was to improve the efficacy of aboveground and foliar applications of entomopathogenic nematodes (EPNs). Improvement in the efficacy of EPNs against foliar insect pests can be accomplished by protecting EPNs from desiccation and ultraviolet light (UV). Barricade\* gel is a desiccation protectant used for fire protection. Shapiro-Ilan et al. demonstrated that Barricade\* gel is effective in improving EPN control of insect pests. The UV radiation absorbing chemicals P-amino benzoic acid (PABA) and octyl methoxycinnamate (OMC) were evaluated in combination with Barricade® gel. The EPN Steinernema feltiae and mealworm larvae (Tenebrio molitor) were used in a series of laboratory experiments to evaluate their ability to protect and enhance EPN infection of insects. None of the chemicals were toxic to EPNs or mealworm larvae. EPNs (100 IJ/ml) were mixed into solutions of 1% v:v PABA + 3% v:v Barricade, 1% v:v OMC + 3% v:v Barricade, 3% v:v Barricade alone, and a water control, poured into 60-mm-d petri plates, and left uncovered while exposed to UV light (Eye Hortilux PowerVeg-T5, full spectrum of UVA and UVB) in a biosafety cabinet for 0, 6, and 12 hrs. After the desired exposure time (0, 6, or 12 hrs), the solution was transferred into a spray bottle and applied onto a 60-mm-d Whitman #1 filter paper that was placed into a petri plate. Three mealworm larvae were introduced into each plate to assess nematode infectivity. In a second experiment, similar protective solutions were evaluated on EPN exposed to UV radiation but applied to a detached leaf of Brassica chinensis var. parachinensis (choy sum) that was placed in a 60- mm-d petri plate. In both experiments, after 48 hrs exposure to the treated EPNs, the number of dead mealworm larvae in a petri plate was determined by a movement response. The OMC+Barricade and PABA+Barricade combinations afforded the greatest protection to EPN in the filter paper and detached leaf experiment. EPN protection from desiccation and UV radiation with chemicals enhanced infection of mealworm larvae in the laboratory. Further experiments are needed to document similar protection under natural conditions.

PROFICIENCY OF INDIVIDUAL OR PAIRED ROOT-KNOT NEMATODE JUVENILES IN LOCATING AND PENETRATING SUS-CEPTIBLE PLANT ROOTS. Adamo, Noah<sup>1</sup>, C.S. Johnson<sup>1</sup> and J.D. Eisenback<sup>2</sup>. <sup>1</sup>Virginia Tech Southern Piedmont AREC, Blackstone VA 23824. <sup>2</sup>Virginia Tech Dept. of Plant Pathology, Physiology, and Weed Science, Blacksburg, VA 24061.

We hypothesized that motile root-knot nematode (*Meloidogyne* spp.) second stage juveniles (J2s) would vary in their ability to locate and penetrate roots of susceptible hosts and that the presence of two individuals might increase the likelihood of success. Laboratory bioassays were conducted from 2017-2019 with two populations of *M. arenaria* from Virginia tobacco fields and two levels of inoculum frequency, individual or paired juveniles. Nematodes were manually deposited into microplots containing seedlings of susceptible tomato (*Solanum lycopersicum*) "Rutgers" in three cubic centimeters of sterile growing medium and maintained at ambient temperature with a 16/8 light/dark cycle for 48-70 hours. Seedlings were destructively sampled and roots stained with acid fuschin to determine whether penetration had occurred. In total, 85 individuals and 60 pairs of juveniles were evaluated in four and three tests, respectively. Only 7 individuals (8.2%) successfully located and penetrated roots in this study, while 21 nematodes that were inoculated in pairs (17.5%) were able to locate and penetrate roots. However, of these paired inoculations, in only 4 cases (6.6%) were both inoculated nematodes successful. Additional bioassays investigating the ability of 55 individual *M. enterolobii* juveniles to enter roots of susceptible tomatoes had similar results, with only 3 individuals (5.5%) successfully penetrating roots. These bioassays confirm the hypothesis that the proficiency of individuals to initiate penetration of susceptible plant roots is variable and generally low in the populations that we investigated, while suggesting that the presence of another nematode increases success when root-knot nematodes are highly dispersed or present at low inoculum densities at the root-soil interface.

## HOST STATUS OF COVER CROPS FOR TWO ECONOMICALLY IMPORTANT ROOT-KNOT NEMATODE SPECIES OCCURRING

IN VIRGINIA. Ahmed, Saleh, and H. L. Mehl. Virginia Tech Tidewater Agricultural Research and Extension Center, Suffolk, VA 23437. Meloidogyne incognita and M. hapla are two root-knot nematode (RKN) species that cause economic losses of agronomic crops in Virginia. M. incognita contributes to yield losses in cotton, corn, and soybean, whereas M. hapla impacts peanut and soybean. Planting winter cover crops has become a common practice in Virginia to reduce soil erosion and enhance soil health. A diversity of cover crop species are used including legumes, brassicas, and grasses, but the ability of RKN populations in Virginia to reproduce on these species has not been determined. A greenhouse experiment was conducted to evaluate M. incognita and M. hapla reproduction on nine cover crops: wheat (Triticum aestivum), barley (Hordeum vulgare), rye (Secale cereal), Austrian winter pea (Pisum sativum subsp. arvense), crimson clover (Trifolium incarnatum), rapeseed (Brassica napus), tillage radish (Raphanus sativus var. longipinnatus), hairy vetch (Vicia villosa), and oats (Avena sativa). Tomato was included in the experiment as a positive control for RKN reproduction. Cover crop seed was planted in cone-tainer cells (150 g) and the greenhouse was maintained at a temperature of  $24 \pm 2$  °C. Half of the plants were inoculated with M. incognita (7,000 eggs/plant) and half were inoculated with M. hapla (6000 eggs/plant). After 60 days, eggs were extracted from the roots and quantified. Barley, oats, and tillage radish were poor hosts for both RKN species. Wheat, rye, and rapeseed were poor hosts for M. incognita. Egg production by M. hapla was ten times higher than by M. incognita on all host plants. For M. hapla, the highest reproduction factor (Rf) was observed on crimson clover (Rf=150) followed by Austrian winter pea (Rf=18) and hairy vetch (Rf=15). For M. incognita, crimson clover was the only cover crop that supported a reproduction factor over 1 (Rf=9). Among the nine cover crops evaluated, crimson clover supported the highest level of reproduction by both RKN species. Crimson clover is often recommend in cover crop mixes due to its nitrogen-fixing properties. However, based on the results of this study, crimson clover should be avoided as a cover crop in fields infested with RKN. Future studies will evaluate reproduction of other economically important plant parasitic nematodes on winter cover crops and the impacts of winter/early spring soil temperatures on nematode reproduction prior to planting of the cash crop.

## MOLECULAR CHARACTERIZATION AND IDENTIFICATION OF ROOT-LESION NEMATODE SPECIES FROM CORN FIELDS IN NORTH DAKOTA. Akhter, Nasima and G. P. Yan. North Dakota State University, Department of Plant Pathology, Fargo, ND 58108.

Root-lesion nematodes (RLN), Pratylenchus spp., are one of the major plant-parasitic nematodes in agriculture and known as one of the most detrimental nematode pests in corn in the U.S. There are more than 80 species of RLN that have been recorded thus far. Identification and characterization of these species are important to obtain effective management strategies. The migratory-endoparasitic nature, wide host range, and high species variation within the genus make their species identification more challenging through traditional methods. Fortunately, molecular tools provide an opportunity to make the species identification and characterization more precise and expeditious. Previous nematode surveys in other crops detected four species of RLN (Pratylenchus neglectus, P. scribneri, and two new Pratylenchus spp.) in North Dakota (ND). Therefore, it was imperative to identify species of RLN in corn fields of ND, characterize them by analyzing their DNA sequences, and determine the phylogenetic relationship among the species. To achieve the objectives, soil samples were collected arbitrarily from 50 corn fields of ND in 2018. Out of these samples, 16 samples (32%) were found positive for RLN after nematode extraction by sugar centrifugal flotation method. The RLN densities varied from 100 to 3,000 nematodes/kg of soil. The positive samples of RLN were identified to species level by species- specific PCR and DNA sequencing of three genomic regions including D2-D3 of 28S rRNA, internal transcribed spacer (ITS) of rDNA, and cytochrome oxidase subunit one (COI) of mtDNA. Out of the 16 samples, six were identified as P. scribneri, four were new Pratylenchus sp.-2016 isolates, three were new Pratylenchus sp.-2017 isolates, and one was P. neglectus. Interestingly, two of the fields that had higher densities of RLN were found to contain a mixed population of P. neglectus and P. scribneri. The neighbor-joining trees for the three regions were constructed independently to better understand the phylogenetic relationship of these species. In the three phylogenetic trees, four species were clustered in four separate clades indicating the divergence among species. Among the four species, P. scribneri and new Pratylenchus sp.-2016 isolates were more closely associated with each other. The new Pratylenchus sp.-2017 isolates were also closely related to the new Pratylenchus sp.-2016 isolates but P. neglectus was not closely associated with other three species. The lowest intra-species variation (0.52 to 3.16%) was found in the new Pratylenchus sp.-2017 isolates whereas the highest intra-species variation (11.93 to 24.21%) was found in the P. neglectus isolates. Molecular characterization of these four species will be helpful for understanding the evolutionary relationships of RLN and for facilitating species identification and management of RLN in infested corn fields.

STRUCTURAL COMPOSITION OF SOIL MICROBIAL COMMUNITIES DO NOT SHIFT BASED ON NEMATODE MANAGEMENT METHOD **Alake, Gideon<sup>1</sup>, P. Timper<sup>2</sup>, D. L. Wright<sup>3</sup>, L. W. Duncan<sup>4</sup>, W.T. Crow<sup>1</sup>, H.T. Alborn<sup>5</sup>, T. Mekete<sup>6</sup>, P. DiGennaro<sup>1</sup>. <sup>1</sup>Dept. of Entomology and Nematology, University of Florida, FL 32611. <sup>2</sup>USDA ARS, P.O. Box 748, Tifton, GA 31793. <sup>3</sup>Dept. of Agronomy, North Florida Research and Education Center, University of Florida, Quincy, FL 32351. <sup>4</sup>Entomology and Nematology Dept., Citrus Research and Education Center, University of Florida, FL 33850. <sup>5</sup>USDA ARS, 1600/1700 Southwest 23rd Drive, Gainesville, FL 32608. <sup>6</sup>Division of Plant Protection, Institute of Food Production and Sustainability, NIFA, 800 9th Street SW, Washington DC.** 

The soil is a living system that acts as a critical biodiversity reservoir and is an essential basis of sustainable agriculture. Soil microbiota plays a crucial role in plant growth and nutrition, and pest management in agricultural production may influence soil biodiversity and shift the structure of soil microbial communities. Peanut, an economically important crop in the southeastern United States, faces an increasing threat from nematode pests. Current nematode management efforts primarily rely on chemical nematicides and resistant cultivars. Using high-throughput Illumina MiSeq, we evaluated structural responses of soil bacteria and fungi to nematode management approaches in peanut agroecosystems. We sampled fields where Telone II was used as a chemical nematode intervention, and fields that used non-chemical practices, such as cover cropping. We also sampled adjacent non-tilled soils to provide a baseline of the soil biodiversity for comparison. There were apparent differences in the composition of bacterial and fungal communities between chemical and non-chemical treated fields. The alpha-diversity was not statistically different between these treatments. Also, pre- and post-plant surveys did not show a measurable difference in microbial diversity. Our results suggest that the current chemical nematode management practice does not quantitatively reduce the diversity of bacteria and fungi. Increased sequencing resolution and improved primer design may significantly enhance our ability to detect specific bacteria or fungal structural shifts in response to nematode management.

#### DEVELOPING REAL-TIME PCR ASSAYS TO DETECT AND QUANTIFY *PRATYLENCHUS SCRIBNERI* DIRECTLY IN DNA EX-TRACTS FROM FIELD SOIL AND POTATO ROOTS. **Arora, Deepika and G. P. Yan.** North Dakota State University, Department of Plant Pathology, Fargo, ND 58108.

Root-lesion nematodes (Pratylenchus spp.) affect crop production worldwide and several species including P. scribneri are detrimental to potato. Pratylenchus scribneri is a migratory endo-parasitic nematode which can infect potato plants and cause poor growth, yellowing of the foliage, and darkened necrotic lesions on roots. After its initial detection in 2016 from a potato field in North Dakota, P. scribneri was found in other fields in seven counties in North Dakota and one county in Minnesota. Till date, there are no published procedures in the U.S. for detecting and quantifying this nematode using DNA extracted directly from field soil or potato roots. Due to its high virulence and wide host range, it is prudent to have a fast, sensitive and specific molecular diagnostic method. Here we report on the development of SYBR Green I-based qPCRassays to detect and quantify P. scribneri in field soil and potato roots. The pair of primers used in this studywas designed from the ITS region of the 28S rDNA. Specificity of primers was evaluated by in silico analysis using 26 other important species of Pratylenchus genus and through qPCR using DNA extracted from P. scribneri along with 18 non-target nematodes and nematode communities, with no resulting cross reactions. Standard curves for soil and root assays were obtained by inoculating sterilized soil or non-infected potato roots with varying number of P. scribneri followed by DNA extraction and qPCR. The standard curves were supported by high amplification efficiency (E) and R<sup>2</sup> values between the numbers of *P. scribneri* artificially added to soil or roots and the numbers quantified using qPCR (E = 94%,  $R^2$  = 0.996 for soil assay; E = 103%,  $R^2$  = 0.988 for root assay). To evaluate the minimum amount of DNA template that could be detected and quantified by the qPCR assays, a serial dilution of template DNA derived from sterilized soil or potato roots inoculated with a single P. scribneri individual was made. The dilution series ranged from one nematode down to an equivalent of 1/512 of a nematode and the assays could detect 1/128 equivalents of the DNA of one nematode. The developed qPCR assays were evaluated by comparing the numbers of target nematode in 20 randomly selected field soil samples and 20 root samples, determined by traditional microscopic counting, with the numbers determined by qPCR. High correlations ( $R^2 = 0.77$  for field soil samples;  $R^2 = 0.73$  for plant root samples) observed between the numbers from the traditional and molecular methods suggest that the assays could be used to enumerate P. scribneri numbers directly in infested field soils and potato roots. Accurate detection and quantification of P. scribneri in fields are critical for designing effective measures to control this nematode pest. The assays developed in this study are straightforward and require no expertise in nematode taxonomy and morphology, and may serve as a valuable molecular diagnostic tool for management of P. scribneri.

FROM PARASITES TO FREE-LIVING WORMS: THE SEARCH FOR MOLECULAR UNDERPINNINGS OF CHEMOTAXIS BEHAV-IORS. **Baiocchi, Tiffany<sup>1</sup>, N. Mercado<sup>1</sup>, K. Kin<sup>2</sup>, B. Strickhouser-Monzon<sup>2</sup>, K. Anesko<sup>2</sup>, P. Robles<sup>2</sup>, A. Dillman<sup>3</sup>. <sup>1</sup>Biochemistry Dept., University of California Riverside, Riverside, CA 92521. <sup>2</sup>Biology Dept., University of California Riverside, CA 92521. <sup>3</sup>Nematology Dept., University of California Riverside, Riverside, CA 92521.** 

Entomopathogenic nematodes (EPNs) are a special group of nematodes that infect and kill insect pests and are used as biological control agents. Our work identified the odor 3-methly-2-buten-1-ol (more commonly known as prenol), which is associated with insect cadavers that have been infected by EPNs and are resource-deficient. Prenol induces repulsion in EPN species, but surprisingly elicits attraction in the free-living species and model organism *Caenorhabditis elegans*. Through use of this odor and a newly-emerged resource: The *C. elegans* Natural Diversity Resource, we have leveraged natural variation in wild isolates of *C. elegans* with genome wide association techniques to identify at least two genes that influence chemotactic behavioral responses to prenol. The goal of this work has been to improve our understanding of nematode behaviors at the molecular level and leverage these findings to better understand what may drive behaviors in EPN species.

MOLECULAR INSIGHTS INTO RESISTANCE RESPONSE TO *MELOIDOGYNE CHITWOODI* RACE 1 AND ITS BREAKDOWN BY AN EMERGING PATHOTYPE ROZA IN POTATO. Sapinder Bali<sup>1</sup>, Kelly Vining<sup>2</sup>, Cynthia Gleason<sup>3</sup>, Hassan Majtahedi<sup>4</sup>, Charles R Brown<sup>4</sup>, Vidyasagar Sathuvalli<sup>1</sup>. <sup>1</sup>Hermiston Agricultural Research and Extension Center, Oregon State University, Hermiston, Oregon 97838, USA. <sup>2</sup>Department of Horticulture, Oregon State University, Corvallis, Oregon 97330, USA. <sup>3</sup>Department of Plant Pathology, Washington State University, Pullman, Washington 99164, USA. <sup>4</sup>Retired from United States Department of Agriculture, Prosser, Washington 99350, USA.

Meloidogyne chitwoodi (aka Columbia root-knot nematode) is one of the most damaging pest of potato in the Pacific Northwest. It infects both potato roots and tubers, causing internal as well as external defects to the tubers rendering them unfit for commercial use hence, causing heavy loses to the potato growers in the region. Host resistance is currently viewed as one of the most effective ways to control the nematode damage. Currently, no commercial potato variety is known to harbor M. chitwoodi resistance. M. chitwoodi Race 1 (MC1) resistance was identified in a wild, diploid species Solanum bulbocastanum (SB22), which was later introgressed into a tetraploid selection, PA99N82-4. However, an emerging pathotype of MC1 designated as MC1Roza successfully breaks this resistance and parasitizes SB22. In the present study, we used RNAseq to evaluate the mechanism of introgressed resistance and its breakdown by MC1Roza. Differential gene expression between PA99N82-4 and a susceptible cultivar 'Russet Burbank' at four time points suggests that nematode attack triggers both PAMP-triggered immunity (PTI) and effector-triggered immunity (ETI) in the resistant clone, which might be inhibiting the feeding site formation. The data suggests an indication of reactive oxygen species (ROS) activity and onset of hypersensitive response (HR) playing significant role in the resistance mechanism. Similar analysis was performed using SB22 inoculated with MC1 and MC1Roza juveniles at six time points to decipher the resistance breakdown mechanism. The highest number of genes (fold change  $\geq 2$ ) were differentially expressed at 48 hours (1062), followed by 14 days (884), 21 days (533), 5 days (35), 10 days (26) and 72 hours post inoculation (04). Gene Ontology analysis showed enrichment for biological processes, cellular component and molecular functions at 48 hours and 14 days post inoculation and only for biological processes, and molecular functions at 21 days post inoculation. The functional annotation and validation of differentially expressed genes is still in progress. This study provides molecular insights into MC1 resistance mechanism and its breakdown by MC1.

COMPARATIVE GENOMICS OF NATIVE STEINERNEMATIDS AND ASSOCIATED BACTERIAL SYMBIONTS FROM FLORIDA CITRUS GROVES. **Baniya, Anil<sup>1</sup>, L.W. Duncan<sup>2</sup>, P. DiGennaro<sup>1</sup>.** <sup>1</sup>Dept. of Entomology and Nematology, University of Florida, FL 32611. <sup>2</sup>Entomology and Nematology Dept., Citrus Research and Education Center, University of Florida, Lake Alfred, USA.

Citrus root weevil (*Diaprepes abbreviatus*) is one of the most significant contributors to yield loss in Florida citrus. Native entomopathogenic nematodes (EPN) in the genus *Steinernema* provide a certain amount of natural biological control of citrus root weevil and have higher persistence upon application. Florida citrus is grown in two major soil ecosystems that dictate the occurrence of different *Steinernema* are also exemplified by species- specific mutualistic associations with *Xenorhabdus* bacterial species. There is a gap in our understanding of EPN and associated bacterial genotypic diversity within Florida citrus groves, therefore it is crucial to understand the diversity of bacterial and nematode genotypes to inform biological control strategies. The two most abundant species of EPN in Florida citrus are *S. diaprepesi*, and *S. khuongi*. We evaluated the genotypic diversity of bacterial isolates from *S. diaprepesi* and *S. khuongi* from multiple citrus groves within different soil ecosystems using nanopore sequencing technology. This platform allows for the quick and economic sequencing of multiple whole bacterial genomes in a single sequencing run. Our goals are to identify genotypic markers for bacterial symbiont specificity, virulence to citrus root weevil, and prevalence of sub-species level bacterial genotypes in different soil habitats. Importantly, the bacterial associate of *S. khuongi* is still unknown. Comparative genomics between the bacterial associates of these two EPN species may shed light on the genetic components informing habitat preferences, host range and insect virulence.

ANALOGS OF PLANT ESSENTIAL OILS AS POSSIBLE NEMATICIDES. **Barizon, Jefferson<sup>1</sup>, J. S. Klimavicz<sup>2</sup>, J. R. Coats<sup>3</sup>, and G. L. Tylka<sup>4</sup>. <sup>1,4</sup>Department of Plant Pathology and Microbiology, Iowa State University, Ames, IA 50011, <sup>2,3</sup>Department of Entomology, Iowa State University, Ames, IA 50011.** 

The soybean cyst nematode (SCN), Heterodera glycines, is considered the most damaging pest of soybean in North America, and rootknot nematodes (RKN; Meloidogyne spp.) are widely distributed in agricultural fields worldwide and cause disease in a vast range of plant species. Management of these nematodes relies on multiple strategies such as growing nematode-resistant varieties and nonhost crops and using biological and chemical controls. However, there are no nematode-resistant varieties for many cultivated crops, the effectiveness of existing resistance is eroding in some cases, economically marketable nonhost plants are not available in certain regions where Meloidogyne is prevalent, and the role of chemical control is declining. Thus, new nematode management strategies are needed. We studied the effects of 37 compounds that are synthetic analogs of monoterpenes from plant essential oils on the hatching of SCN. Compounds were dissolved in a solvent containing 0.08% ethanol, 0.08% Triton X-100, and nano-purified water and they were tested at concentrations of 10 and 100 ppm. In continuous exposure assays, SCN eggs were incubated in treatments for 15 days, and in short-term exposure assays, eggs were incubated in treatments for one day, rinsed, then incubated in deionized water for 14 days. The number of hatched second-stage juveniles was determined for both assays after 3, 6, 9, 12, and 15 days. Deionized water, 5.5 mM zinc sulfate, and the solvent were used as controls, and hatching was calculated relative to hatching in the solvent. Eighteen compounds inhibited hatching by 48% to 96%, four of which inhibited hatching at both concentrations and both exposure durations. Two compounds stimulated hatching by 287% to 325% relative to the solvent control, but only at 100 ppm with continuous exposure. Fifteen compounds were further evaluated for the potential to reduce galling by RKN in growth chamber experiments. Cucumber (Cucumis sativus) seeds were planted in cone-tainers containing sterilized sand and grown for 12 days, after which 20 ml of the treatments were applied as a drench followed by infestation with 2,000 eggs of M. incognita in water. Deionized water, 100 ppm abamectin, and solvent were applied to cone-tainers and infested with M. incognita eggs as control treatments. At 15 days after infestation, cucumber roots were washed to remove the sand, and root galling was assessed and compared to galling in the solvent control. The percentage galling for the water, solvent, and abamectin treatments was 39%, 24%, and 0%, respectively. The galling of plants receiving the monoterpene analogs ranged from 16% to 35%, and none of the tested compounds significantly decreased RKN galling compared to the solvent control. The results obtained demonstrate the potential for analogs of plant essential oils to affect hatching of SCN in vitro, but no effects on RKN galling in vivo were detected. Many compounds remain to be tested.

EXPLORING THE CURIOUS RISE AND FALL OF NEMATICIDE USE FOR BROCCOLI IN CALIFORNIA. **Becker, J. Ole<sup>1</sup>, S.T. Koike<sup>2</sup>, Y.Y. Chen<sup>1</sup>, and J. Becker<sup>1</sup>.** <sup>1</sup>Department of Nematology, University of California, Riverside, CA 92521. <sup>2</sup>TriCal Diagnostics, Hollister, CA 95023.

California produces 92% of U.S. broccoli, most of it along the cool central coast area. These coastal fields are regularly rotated with Cole crops. The soil fumigant l,3-dichloropropene (1,3D) was credited for having prevented significant crop damage by plant-parasitic nematodes, foremost for control of the sugar beet cyst nematode, Heterodera schachtii. However, in 1990 the California Department of Food and Agriculture canceled all permits for 1,3D use, as high concentrations of the fumigant were detected in the ambient air of a junior high school. The subsequent unavailability of 1,3D was predicted to result in a significant economic loss to broccoli growers who in that year had treated 1960 hectares with the intent of managing plant-parasitic nematodes. Consequently, in 1993, coastal California broccoli growers greatly increased the use of metam-sodium and particularly fenamiphos (under section 18) to 521 and 9266 treated hectares, respectively. The fenamiphos permit expired in 1998, but 1,3D had become available again two years earlier. However, its use reached less than 15% of the demand six years earlier. By 2010, only 15 hectares were treated with 1,3D and 12.5 hectares with metam-sodium. In 2014, none of the coastal broccoli fields received applications of soil fumigants or contact nematicides. Curiously, the demise of nematicide use had no apparent adverse effect on plant growth. Broccoli yield averaged about 6400 kg/hectare in the 1950s. Since then it has increased by about 250 kg/hectare each year, reaching close to 22000 kg/hectare in 2016. During the same year, a spring survey of 88 broccoli fields along the Central California coast, from Santa Cruz to Santa Barbara counties, found Heterodera cysts in 37.5% of the locations. In the infested areas, the population density varied from 0.5 to 18.5 cysts/250 cm<sup>3</sup> soil and averaged approximately 3.2 eggs/cm<sup>3</sup> soil. Only two field samples contained more than ten cysts/cm<sup>3</sup> with about 250 eggs/cyst. Although economic thresholds have not been established for sugar beet cyst nematodes in California's broccoli production, the overall low infestation density support observations by local university extension personnel and industry field professionals that cyst nematode parasitism and resulting crop damage in California's broccoli crops have rarely been observed. The question will be discussed if the nematode populations diminished because of some change in crop management, the development of an antagonistic microbiome or if the previous treatments were based more on marketing efforts than soil sample data.

HIGH ARCTIC NEMATODE COMMUNITIES OF NORTHWEST GREENLAND. **Bernard, Ernest C.<sup>1</sup>, S.M. Schaeffer<sup>2</sup>, L.S. Taylor<sup>2</sup>, G. Phillips<sup>1</sup>, and J.M. Welker<sup>3,4</sup>. <sup>1</sup>Entomology & Plant Pathology Department and <sup>2</sup>Biosystems Engineering & Soil Science Department, University of Tennessee, Knoxville, TN 37996, <sup>3</sup>Department of Biological Sciences, University of Alaska, Anchorage, AK 99508 and <sup>4</sup>Ecology and Genetics Research Unit, University of Oulu, Finland and UArctic.** 

High Arctic ecosystems (those above 70<sup>o</sup>N) are some of the harshest habitats in the Northern Hemisphere with polar desert and polar semi-desert ecosystems dominating these terrestrial habitats not covered in ice. These very cold, very dry ecosystems have sparse vegetation, low productivity and low soil organic matter. The land surface is only 50% to <10% covered with vascular plants, the remainder being moss or lichen-covered ground or bare soil. Major questions today for these High Arctic ecosystems relate to their composition and biodiversity compared to other extreme environments and how these soil communities will change as the High Arctic warms or becomes wetter in summer. We obtained soils from a long-term project on ecosystem responses to global change at a High Arctic site, Pituffik Peninsula, near Thule, in NW Greenland (76°N 15°E). In 2018, 20 vegetated soil surface samples (0-2 cm depth) were gathered from a randomized block experiment for analysis of their nematode constituents. Treatments consisted of wetted (W) plots (50% more water than naturally occurring), two treatments with increased ambient temperatures (T1: 3°C above ambient; T2: 6°C above ambient), a T2+W treatment, and untreated control. Nematodes were extracted by means of sugar flotation-centrifugation and identified primarily to genus. Thirty-nine taxa were determined among the 20 samples, with an estimated species richness of about 50. Taxon richness per plot ranged from 12-21, while abundance was 316-3830 per 100 cm<sup>2</sup> soil. Fourteen bacteria-feeding taxa were identified; Cephalobidae (5 taxa) were dominant, with Acrobeloides spp. sometimes accounting for more than half of the nematodes in a sample. Rhabditidae (Mesorhabditis sp.) was detected once and Diplogastridae was absent. Eight fungivore taxa were found, with Filenchus spp. the most numerous. Predators were primarily Aporcelaimellus spp., present in nearly every sample. Plant-parasitic nematodes were represented by seven taxa. Food web condition was characterized by low enrichment and low to moderately high structure (quadrants C and D) regardless of treatment. Plots within treatments were not notably aggregated. Abundance, richness, evenness and Shannon diversity were similar among the treatments, but Acrobeloides had a strong positive response (P = 0.02) to the T2+W treatment. Aporcelaimellus exhibited no significant treatment response. These results are similar to those found in the Antarctic Dry Valleys, where an increase of the sole bacterivore did not result in a predator increase. This lack of correlation in Greenland may be due to the long life cycles of predators or to a "reset" of the soil environment during the long winters. In addition, the Greenland nematode communities are far more diverse and complex than those in the Antarctic Dry Valleys, with multiple interactions and outcomes likely among the various species and functional groups.

CONTROL OF *GLOBODERA PALLIDA* USING *BRASSICA JUNCEA* SEED MEAL EXTRACT AND TRAP CROP *Solanum sisymbrii-folium*. **Bhatta, Bhupendra<sup>1</sup>, L.-M.<sup>1</sup>, M. Morra<sup>2</sup>, and I. Popova<sup>2</sup>.** <sup>1</sup>Department of Entomology, Plant Pathology and Nematology, 875 Perimeter Drive MS 2329, University of Idaho, Moscow, ID 83844-2329, <sup>2</sup>Department of Soil and Water Systems, 875 Perimeter Drive MS 2340, University of Idaho, Moscow, ID 83844-2340.

*Globodera pallida* commonly known as pale cyst nematode (PCN), a regulated economically important pest of potato was detected in Idaho in 2006. Eradication of this nematode is a top priority. Individually, trap crop species *Solanum sisymbriifolium* (litchi tomato), and biofumigation by *Brassica juncea* seed meal extract are proven highly effective in controlling PCN. *Solanum sisymbriifolium* is an annual herb that induces egg hatching but does not support nematode development and reproduction. The nematicidal property of *B. juncea* is attributed to 2-propenyl isothiocyanate- a volatile toxic compound produced by hydrolysis of the glucosinolate sinigrin. In this work, we discuss an integrated approach to eradicate *G. pallida* using *S. sisymbriifolium* and *B. juncea* seed meal extract. We hypothesize that the nematodes that hatch in response to *S. sisymbriifolium* cannot complete their life cycle and die, and the remaining population is killed by the activity of sinigrin. We tested the efficacy of different rates of *B. juncea* seed meal extract with or without *S. sisymbriifolium* on hatch

and reproduction of *G. pallida* on potato in greenhouse and field experiments. Here, we present data on the performance of five different rates of *B. juncea* seed meal extract, i.e. 0 lbs/acre, 125 lbs/acre, 250 lbs/acre, 500 lbs/acre and 1000 lbs/acre, to control PCN when used alone versus when combined with *S. sisymbriifolium* in greenhouse experiment. We measured the effect of the treatments on the reduction on cyst content (eggs and juveniles), viability, and hatch of *G. pallida*. Fewer encysted eggs were found after treatment with litchi tomato alone than when in combination with the biofumigation implying that the nematodes hatched in the presence of the trap crop, but that the biofumigant inhibited hatch of the nematode at all rates used. Hatch of the nematode was further reduced when both litchi tomato and *B. juncea* extract were combined. Reproduction assays to determine the viability of the remaining encysted egg population are underway.

# IMPACT OF FLUENSULFONE ON SOIL HEALTH INDICATORS ASSOCIATED WITH FOUR MICHIGAN ECOSYSTEMS. **Bird, George<sup>1</sup>** and **Pablo Navia**.<sup>2, 1</sup>Department of Entomology, Michigan State University, East Lansing, MI48824. <sup>2</sup>ADAMAAgricultural Solutions, Raleigh, NC 27604.

Soil health is an emerging domain of science and agriculture. It is biologically oriented and different from the chemical focus of classical soil science. Relatively little is known about the impacts of agricultural chemical inputs on soil health indicators. The objective of this research was to determine of the impact of Fluensulfone (Nimitz) on thirty soil health indicators associated with four different ecosystems: 1) a corn/soybean/wheat rotation field and 2) adjacent deciduous woodlot, 3) a vineyard and (4) immediately adjacent apple orchard. Nimitz was applied at the agronomic crop and woodlot sites on May 31, 2018, to eight, nine square feet plots at a rate of 3.3 liters per 0.4 hectares in 75 liters of water and immediately irrigated with 6.4 hectare-mm of water. Nimitz was applied at the vineyard and orchard sites on August 24, 2018, using the procedure previously described. As controls, eight plots were treated with 75 liters of water and immediately irrigated with 6.4 hectare-mm of water. Samples for the soil health indicator analyses were taken at the time of Nimitz application and 90 days post-application. The soil health indicators evaluated included water stable aggregates, active carbon, nitrogen mineralization potential, water availability, surface hardness, subsurface hardness, pH, extractable phosphorus, extractable potassium, four minor elements, twelve nematode taxa, endomycorrhizal spores, and micro-oligocheates. The Jenkins centrifugal flotation process was used for nematode, mycorrhizal spore, and micro- oligocheate extractions; whereas, the Cornell University Soil Health Protocol was used for quantification of the other soil health indicators. The four sites had significantly different nematode community structure signatures. For the plant parasites, soybean cyst, root-lesion and lance nematodes dominated the agronomic crop site, while ring and dagger nematodes were most prominent in the adjacent woodlot. Nimitz did not have any detectible positive or negative impact on the soil health indicators evaluated for these two sites. The most apparent difference was that the soil organic matter associated with the agronomic crop site was circa 50% less than that of the adjacent woodlot. In the vineyard and adjacent orchard, Nimitz had no significant (P = 0.05) impact on the suite of sixteen Cornell University soil health indicators. Nimitz, however, provided excellent control of northern root-knot nematode in the vineyard. The Tylenchoid fungivore populations, however, were significantly (P = 0.05) greater in the vineyard control, compared to the Nimitz treatment. This was not true in the adjacent orchard. The Monochoid populations were greater (P = 0.07) in the Nimitz- treated grape plots, compared to the controls. The vineyard appeared to the best test system for the nematicidal properties of Nimitz. At these four locations, Nimitz did not have a detectible impact on the indicators currently commonly being used in the domain of soil health.

EVALUATING SOIL CONDITIONS IN FORMER AGRICULTURAL LAND BEING INCORPORATED INTO VOLCANOES NATION-AL PARK, RWANDA. **Butera, Blanche<sup>1</sup>, R. Higgins<sup>1</sup>, P. Mullin<sup>1</sup>, T. Harris<sup>1</sup>, E. Jeske<sup>2</sup>, R. K. Powers<sup>1</sup> and T. O. Powers<sup>1</sup>. Department of Plant Pathology, 406 PSH, University of Nebraska, Lincoln, NE 68583. <sup>2</sup>Agronomy Dept., University of Nebraska.** 

In this study we compare biotic and abiotic properties of parkland soil to newly acquired farmland soil adjacent to Volcanoes National Park in northern Rwanda. The 27.8 hectares of farmland was used for subsistence farming of 31 potatoes, onions, and climbing beans for approximately 60 years. Eight 40 x 40m grids were established, four in the agricultural land and four within the park boundaries. The soils are Andisols, young volcanic soils with low bulk density and high water holding capacity. The former agricultural soil was determined to have lower concentrations of nitrates, potassium, and magnesium with an average pH of 5.4 versus 6.4 for the forested, parkland soil. The soil microbial community was assessed by fatty acid methyl ester analysis (FAME). The microbial biomass was higher in the forested soils with bacterial biomass nearly double that of the agricultural soils. Differences in the nematode community composition were observed by morphological analysis and by CO1 DNA barcoding. Over 50% of the species in the agricultural soils were classified as plant parasites, with fewer fungivores compared to forested sites. Both parkland and farmland soils had high levels of *Scutellonema paralabiatum*, but overall nematode diversity and plant-parasitic nematode diversity was higher in agricultural sites. *Globodera rostochiensis* and *Meloido-gyne hapla* were exclusively found in agricultural sites. Among the taxa found exclusively in forested sites were *Alirhabditis* sp., a microbivore, *Neopsilenchus minor*, a fungal feeder, two predators, *Aporcelaimellus obtusicaudatus* and *Eudorylaimus aquilonaris*, and three plant parasites, *Ogma brevistylus*, *Longidorus intermedius*, and *Xiphinema rotundatum*.

INOCULATION OF *LITYLENCHUS CRENATAE* TO AMERICAN BEECH SEEDLINGS AND RECOVERY OF NEMATODES. **Carta, Lynn<sup>1</sup>, G. Bauchan<sup>2</sup>, D. Burke<sup>3</sup>, J. Koch<sup>4</sup>, D. Martin<sup>5</sup>, S. Reed<sup>6</sup>, and Q. Yu<sup>7</sup>. <sup>1</sup>USDA-ARS Beltsville, MD. <sup>2</sup>Electron and Confocal Microscopy Unit, USDA-ARS, Beltsville, MD 20705. <sup>3</sup>Holden Arboretum, Kirtland, OH. <sup>4</sup>USDA-FS, Delaware, OH. <sup>5</sup>USDA-FS, Morgantown, WV. <sup>6</sup>Ontario Forest Research Institute, MNRF, Sault Ste. Marie, Ontario, Canada. <sup>7</sup>Agriculture and Agrifood Canada, Ottawa, Ontario, Canada.** 

Beach Leaf Disease (BLD) was first detected in northern Ohio in 2012 and can kill trees within seven years of detection, but until recently the causal agent was unclear. Recently Koch's postulates were successfully completed with the nematode *Litylenchus crenatae* on greenhouse seedlings of American beech, *Fagus grandifolia*, in Ohio, USA and Ontario, Canada. Symptoms associated with BLD were reproduced, and the nematode and its internal transcribed spacer region of ribosomal DNA (ITSrDNA) were re-isolated from symptomatic tissue. Nematodes were inoculated in the fall of 2018 to beech tree seedlings in three locations (Kirtland and Delaware, OH and Sault Ste. Marie, Ontario, Canada) of either primarily adults or of mixed stages (eggs, juveniles and adults) to either leaves, buds, or both, with and without mechanical injury from a needle. Multiple nematode life stages other than males were found within buds, and males were found within leaves. All types of inoculation produced symptoms of crinkling and dark green to chlorotic banding on the leaves. The combination of injury plus mixed stage nematodes to buds was most consistently effective in producing symptoms. Despite the fact that the DNA of the nematode population originally found in Ohio (and now PA, NY and Ontario, Canada) matched the ITS rDNA sequence of *Litylenchus crenatae* found in Japan, the U. S. nematode population on the grounds of the Holden Arboretum has not affected *Fagus crenata* (its Japanese host) living among infested *Fagus grandifolia*. Neither has the Japanese population been reported in association with *F. grandifolia* or *F. sylvatica* in Japan. The damage associated with the nematode in North America is more severe than in Japan and appears to be spreading throughout the region around Lake Erie. The disease virulence, transmission and control are subjects of ongoing studies by various other labs in the region.

NEMATAIL REMAINS: HOW THE PREDATORY MITE, *STRATIOLAELAPS SCIMITUS*, ATE FOUR SOIL NEMATODE SPECIES. **Carta, Lynn<sup>1</sup>, G. Bauchan<sup>2</sup>, J. Mowery<sup>2</sup>, R. Ochoa<sup>3</sup>, and E. Palevsky<sup>4</sup>.** <sup>1</sup>MNGDBL, USDA-ARS, Beltsville, MD 20705. <sup>2</sup>Electron and Confocal Microscopy Unit, USDA-ARS, Beltsville, MD 20705. <sup>3</sup>Systematic Entomology Laboratory, USDA-ARS, Beltsville, MD 20705. <sup>4</sup>Newe-Ya'ar Research Center, Agricultural Research Organization, Ministry of Agriculture, Ramat Yishay 30095, Israel.

The omnivorous, predatory mite Stratiolaelaps scimitus is a commercially available biocontrol agent used against various greenhouse pests including gnat, housefly, thrips, springtails and other soil-borne larvae. Recently this soil-litter inhabiting mite was reared on the free-living, bacterial-feeding nematode Rhabditella axei to improve its performance in reducing the galling caused by plant-parasitic Meloidogyne incognita nematodes attacking tomato in greenhouse pots in Brazil. However, the microscopic feeding habits of predatory mites on nematodes are poorly understood. Therefore, starved S. scimitus female mites were presented with selected soil nematodes (plant-parasitic tylenchids, Meloidogyne incognita, Heterodera glycines, and free-living bacterial-feeding rhabditids Pristionchus aerivorus and Mesorhabditis inarimensis and fungal-feeding Aphelenchoides bicaudatus and Bursaphelenchus sp. reared on Rhizoctonia solani). This research was conducted in staged arenas of plastic-domed metal cups, each containing at least six mites and a brass plate covered with filter paper sprinkled with some small pieces of vermiculite. Dozens to hundreds of nematodes in water were pipetted into the center of each filter paper, mite feeding was observed, and videos were made of mites attacking, maneuvering and eating nematodes under the Hirox digital microscope (Hirox-USA,Inc., Hackensack, NJ). Brass plates containing filter paper and the mites feeding on nematodes/ were then plunged into liquid nitrogen and observed under the low-temperature SEM (Hitachi 4700, Hitachi America, Ltd., Dallas, TX, with a Quorum PP2000T, (Quorum Technologies Ltd., East Sussex, UK). Mites snatched individual nematodes at their mid-body with palps, rolled them compactly like spaghetti on a fork, and punctured the cuticle with chelicera so that liquid gut content flowed into the long cheliceral trough toward the more ventral sucking mouthparts. This was followed by back and forth cleaning of chelicera before the next worm was snatched. The whole process lasted approximately three minutes. The tail was often prominently visible as the mite ate the nematode. Mites ignored recently killed M. inarimensis that had been frozen at - 20° C for ten minutes, but devoured actively moving M. inarimensis. While other tested species were not frozen like this, Aphelenchoides and Bursaphelenchus were not consumed by these mites, possibly due to reduced nematode activity or to their fungal diet. The latter was confirmed in a recent study by the fourth author on another soil mite predator Macrocheles embersoni, where individuals of the nematode Rhabditella axei fed on yeast were nutritionally inferior to those fed on bacteria. These observations complement our potted plant results above and further demonstrate the potential of soil mite predators as candidate biocontrol agents for the control of soil nematode pests.

### HOST STATUS AND SUSCEPTIBILITY OF DIFFERENT HEMP (*CANNABIS SATIVA* L.) CULTIVARS FOR ROOT-KNOT NEM-ATODE (*MELOIDOGYNE JAVANICA*). Coburn, Jacqueline, J. Desaeger. Department of Entomology and Nematology, University of Florida Gulf Coast Research and Education Center, 14625 CR 672, Wimauma, FL 33598.

The 2018 Farm Bill and a pending 2019 Florida Statute removed hemp from the controlled substances list, and redefined industrial hemp (Cannabis sativa) as an agricultural commodity (textiles, papers, fabrics, insulation, hempseed oil). Farmers in Florida are enthusiastic, but very little is known about how hemp will grow in the state. Nematodes, especially root-knot (Meloidogyne spp.) and sting nematodes (Belonolaimus longicaudatus), are one of the main limiting factors to crop production in Florida and understanding the nematode host status of hemp will therefore be critical. A greenhouse study was set up at the Gulf Coast Research and Education center (GCREC) of the University of Florida to explore the host status and susceptibility of six different hemp cultivars (Helena, Tygra, Fibranova, Eletta Campana, Carmagnola, and Carmagnola Selezionata) to Meloidogyne javanica, one of the most common root-knot nematodes in Florida. Cultivars are evaluated with and without nematodes in pots filled with steamed soil. Seeds were presoaked in distilled water for one hour before being placed in a moisture chamber, and four days later germinating seeds were planted in the pots. Each cultivar had ten replicates-five with nematodes and five without. The replicates with nematodes had two plants potted per pot, of which one will be sampled after one week and stained for nematodes. In addition to the hemp, five extra pots were seeded with cucumber and inoculated to serve as an inoculum check. Pots were inoculated with 10,000 root-knot nematode eggs three days after planting. Germination ranged from 62% to 74% between cultivars with Tygra having the highest rate. One week after inoculation with root-knot nematode eggs, hemp roots were stained for evidence of root-knot nematode infection. Differences in juvenile presence within the roots varied amongvarieties. Cucumber roots averaged 45 juveniles per root system while the average for the hemp ranged from 6 to 60 juveniles between cultivars. Nematode effects on hemp growth will be measured by evaluating each cultivar for height, root galls, dry plant weight, and the amount of eggs in the root system two months after inoculation. Based on the host status and susceptibility, recommendations can be made for aide in selecting the more appropriate cultivar for hemp field use in Florida.

COMPOSTS AND MANURES FOR POTATO EARLY DIE CONTROL Emilie Cole and M. Quintanilla. Michigan State University, Department of Entomology, 288 Farm Ln. Room 26, East Lansing, MI 48823.

The interaction of root lesion nematodes (*Pratylenchus penetrans*) and *Verticillium dahliae* causes potato early die complex (PED) which induces premature vine senescence and dramatically reduces yield. Management is often achieved through the use of soil fumigants and nematicides, but their adverse effects on soil, human and environmental health and strict regulations worldwide require alternative control tactics. In this study, we investigated the effect and mechanism of poultry manure and two compost blends (dairy doo and layer ash blend) in controlling PED. In a lab assay, 200 root lesion nematodes were exposed to poultry manure, layer ash blend, dairy doo, or wood ash, for 7 days at rates of 0, 0.1, 1, 10, and 20 percent product and assessed for nematode survivorship. Additionally, these products were evaluated for volatile fatty acid content to elucidate mechanisms. Composts and manures were tested in the field at two rates, high (11.2 t/ha) or low (2.8 t/ha), and populations of *P. penetrans* and *V. dahliae* were evaluated. Poultry manure and layer ash blend showed the greatest nematode control in lab assays with 24.5% and 38.2% reduction at 1% compost application, respectively with decreased populations at higher rates. In the field, plots treated with poultry manure at a high rate had significantly fewer nematodes, while layer ash low had the lowest *V. dahliae* germination in collected soil. Volatile fatty acid content in the layer ash blend and poultry manure suggest it could be a mechanism for the reduction in PED pressures observed.

LANCE NEMATODES, AN INCREASING PROBLEM ON GOLF COURSES IN THE SOUTHEASTERN UNITED STATES. **Crow, William T.,** Entomology and Nematology Department, University of Florida, Gainesville FL, 32611. Golf course turfgrasses in the southeastern United States can suffer damage from many different genera of plant-parasitic nematodes (PPN). However, the majority of this nematode damage is attributed to three genera; *Belonolaimus* (sting nematode), *Meloidogyne* (root-knot nematode), and *Hoplolaimus* (lance nematode). In past decades fenamiphos was widely used for nematode management on golf courses in the region. Fenamiphos has both contact and systemic activity and generally provided acceptable results on all the nematodes of concern. In recent years fenamiphos has been replaced on turf largely by three active ingredients, abamectin, fluensufone, and fluopyram. These nematicides can provide excellent results on sting and root-knot nematodes, but for several reasons none of them provide consistent efficacy on lance nematode. The frequency of detection of lance nematode on golf courses in the southeast has increased, and the population densities of lance nematodes in these detections has risen since 2015, the year use of these new nematicides became common. In 2018 the percentage of golf course samples from Florida submitted to the University of Florida Nematode Assay Lab exceeding existing risk thresholds for lance nematode was 79%, up from a 43% average for the proceeding decade. We hypothesize that these increases result from decreased competition for food resources by other PPN that are more differentially impacted by the current nematicides used.

REDUCTION OF THE ROOT LESION NEMATODE, *PRATYLENCHUS PENETRANS*, IN CARROT USING NEMATICIDE APPLICA-TIONS. Elisabeth Darling, K. Poley, and M. Quintanilla. Department of Entomology, Michigan State University, East Lansing, MI 48823.

Over the span of three growing seasons, 12 nematicide treatments, both chemically- and biologically-based, have been evaluated on processing carrots to determine their effectiveness on reducing an economically important plant- parasitic nematode, Pratylenchus penetrans, the root lesion nematode (RLN). The RLN has a very wide host range including many vegetables, field crops, and common weeds. There is an increasing demand for an effective and safe method to manage populations of RLN on carrots. Feeding by RLN can produce unmarketable characteristics such as stubby, hairy, or forked carrots, ultimately resulting in yield loss. A field trial was established in Hart, Michigan on processing carrots, variety Cupar, to determine varying nematicides' effectiveness against the RLN in 2017. Field sites were sampled to determine level of infestation. Six nematicide treatments were tested (Vydate, Majestene, Melocon, two application rates of Nimitz, and Nematec). Each treatment, including an untreated control, was replicated five times. Soil samples were collected three times throughout the growing season: at planting, mid-season and harvest. At harvest, a one- meter row of carrots was randomly selected from each plot and graded based on USDA standards. We hypothesized that (1) the selected treatments would greatly reduce the population of plant-parasitic nematodes and decrease unmarketable characteristics; (2) beneficial nematodes would be more negatively impacted by chemical treatments. The 2017 field trial showed that Vydate, the grower standard in Michigan, had the highest marketable yield, and Melocon (a bionematicide) had the highest number of forked carrots. However, there were no significant differences in plant-parasitic or beneficial nematode counts among treatments. The following year in 2018, a field trial on a different plot in Hart, MI was established using results from 2017 as a base for treatment selection. Seven treatments were applied: Vydate, Majestene, Melocon, Nimitz, LAB Blend compost, Velum Prime, ADA 36380. Methods from 2017 were kept consistent and soil sampling occurred 21 days post-planting, mid-season, and harvest. Results between post-planting and mid-season sampling were not significant but indicated that root lesion nematodes declined in all treatments except Melocon, Majestene, and ADA. Root lesion nematodes appeared to decline the most in Compost and Vydate. Additionally, beneficial nematodes decreased insignificantly in Velum Prime, ADA 36380, and Nimitz. The LAB Blend Compost appeared to be the most effective at reducing root lesion nematode populations. Beneficial nematode populations generally increased or remained unchanged in biological treatments (LAB Blend Compost, Melocon, and Majestene). Results for harvest 2018 are pending. A 2019 field trial using the following six treatments is ongoing, based on the combined results of 2017 and 2018: Vydate, Melocon, Majestene, LAB Blend Compost, Vydate + LAB Blend Compost, and Majestene + LAB Blend Compost.

A PASTEURIA '*EYE VIEW*' OF THE NEMATODE CUTICLE. **Davies, Keith G.,** Department of Biological and Environmental Sciences, University of Hertfordshire, Hatfield, AL10 9AB, United Kingdom.

Several species of endospore forming bacteria belonging to the *Pasteuria* group that are natural enemies of plant- parasitic nematodes have long been known to have potential to be developed into biological control agents. One of the current major constraints concerning their commercial development is their host specificity. Field populations of plant- parasitic nematodes usually occur as mixed populations and isolates of *Pasteuria* will adhere to one sub-population of phytonematodes but not another. Therefore, if isolates of *Pasteuria* are to be successfully deployed in the field an understanding of the mechanism by which endospores adhere to the nematode cuticle is essential to ensure that the endospore isolates applied can attach to the nematodes present in the soil. Resent research exploiting genomic sequence of

*Pasteuria* has identified some 17 putative collagen-like proteins expressed on the surface of the endospore and these have been hypothesised to interact with a mucin-like protein present on the surface of the cuticle. Bioinformatic characterisation of these collagen-like proteins shows them to be diverse and possibly obtained through horizontal gene transfer from a variety of sources including viruses. Experiments using endospore adhesion bioassays to characterise the molecular nature of the second-stage juvenile cuticle surface have revealed that although mucin-like proteins may be involved the mechanism is more complex than hitherto thought and will be discussed in detail.

# EFFECT OF SIMULTANEOUS WATER-DEFICIT STRESS AND *MELOIDOGYNE INCOGNITA* INFECTION ON COTTON FRUIT DISTRIBUTION PATTERNS. **Davis, Richard F.<sup>1</sup>, J. L. Snider<sup>2</sup>, H. J. Earl<sup>3</sup>, and P. Timper<sup>1</sup>.** <sup>1</sup>USDA-ARS, Tifton, GA, USA. <sup>2</sup>Dept. of Crop and Soil Sciences, Univ. of Georgia, Tifton, GA, USA. <sup>3</sup>Dept. of Plant Agriculture, Univ. of Guelph, Guelph, Ontario, Canada.

Both water-deficit (drought) stress and Meloidogyne incognita infection reduce cotton yield and fiber quality, and M. incognita damage often causes symptoms similar to drought stress. The reductions in cotton lint yield and quality caused by drought stress and M. incognita infection are additive. To determine whether seedcotton yield reductions due to drought and M. incognita infection result from similar effects on yield components and boll distribution patterns, a seven-year study was conducted in a field infested with M. incognita. A split-plot design was used with the main plot factor as one of three irrigation treatments (non-irrigated, moderate irrigation, and water-replete) and the subplot factor as 0 or 56 l/ha 1,3- dichloropropene. We prevented water-deficit stress in water-replete plots by supplementing rainfall with irrigation. Moderate irrigation plots received half the water applied to water-replete plots. As previously reported, seedcotton yield was strongly affected by year, irrigation treatment and nematicide treatment, but no interactions were observed between irrigation and nematicide, which verified that the yield limiting effects of nematode infection could not be mitigated by providing a water-replete condition. Yield declines due to nematode infection and water deficit were primarily driven by a decline in total boll density (fewer bolls/plant resulting in fewer bolls/ ha) rather than a reduction in the weight of individual bolls. Yield distribution patterns showed that water deficit negatively affected boll numbers for all branch types and fruiting branch positions and that the mainstem node of peak boll distribution was lower under drought resulting in a more compact boll distribution on the plant. This was also the case for nematode infected plants (except that boll density at position two was not affected by fumigation). One notable difference between drought and nematode effects was that the relative decline in boll density due to nematodes was roughly the same for all branches and fruiting sites, whereas the relative decline in boll density for droughtstressed plants was greater at sympodial positions farther from the mainstem. We conclude that the effects of nematode parasitism and water-deficit stress on cotton yield components and boll distribution are generally similar but they are independent and therefore additive.

## NEMATICIDE COCKTAILS FOR IMPROVED EFFICACY IN FLORIDA STRAWBERRIES. Desaeger, Johan, T. Watson and J. Coburn.

Department of Entomology and Nematology, University of Florida, Gulf Coast Research and Education Center, Wimauma, FL 33598. Several new nematicides have recently become available, and more registrations are expected in the next few years. The new nematicides are very different from previous nematicides asthey are moreselective, and they have a safer toxicity profile. However, in Florida their efficacy is often lower compared with soil fumigants, especially when nematode pressure is high. One possible practice to try and improve the efficacy of nematicides is by combining ormixing them. Combinations or cocktails of chemicals have often shown to be more effective at killing pests and diseases than each of the chemicals used separately. This is the case for chemotherapy cocktails in cancer treatments, but has also been shown for antihelmintic treatments in animals and humans, and for fungicide, insecticide and herbicide applications in agriculture. To evaluate the potential of nematicide mixtures, we recently started a series of field trials evaluating various nematicide combinations, either by mixing and applying them together, or by applying different products sequentially. The first trial was a strawberry trial, conducted at the University of Florida's Gulf Coast Research and Education Center in Wimauma, FL between September 2018 and April 2019. Combinations of five nematicides, including two new synthetic products (Nimitz (a.i. fluensulfone) and Velum (a.i. fluopyram)), and three biological products (Majestene, (a.i. Burkholderia rinojensis), Melocon (a.i Purpureocillium lilicanus) and Dazitol (a.i. mustardoilandcapsaicin)wereevaluatedandcomparedtothe fumigant standard K-pam (a.i. metam potassium). The strawberry cv. was Sweet Sensation. Nematode soil samples were collected at planting, at mid-season and at final harvest. Crop vigor was measured bi-weekly, and fruits were harvested twice a week from December 2018 through March 2019. Stand counts and plant vigor showed no differences among treatments. Strawberry yields were greater for the combination of Nimitz followed by Velum followed by Majestene, as compared to the fumigant standard K -pam. Also, Nimitz followed by Majestene and the Velum + Majestene cocktail showed a numerical yield increase. Early and mid -season nematode sampling indicated low to moderate populations of plant-parasitic nematodes, mostly stunt (Tylenchorhynchus spp.) and sting (Belonolaimus longicaudatus) nematodes, with no significant differences between treatments. Bacterial, and especially fungal-feeding nematode groups, were decreased at early and mid-season following the fumigant treatment. End-of-season nematode counts are still being evaluated, and will be reported at the conference.

## THE PROBLEM WITH BIOLOGICAL CONTROLS. DiGennaro, Peter, W. Hu, R. Dyrdahl-Young. University of Florida, Department of Entomology and Nematology, Gainesville FL 32611.

Nematode parasitism is a yield-limiting factor in many cropping systems, translating into substantial economic losses. As potential profits are the major driver of many decisions in the field, it is important we include economic analyses when evaluating nematode management practices. We can determine potential economic returns by calculating the total revenue and subtracting the cost of production, including chemical and biological nematicides. We analyzed the economic returns using stochastic parameters for two biological controls (MeloCon and Majestene), six chemical controls (Mocap, Movento, Nimitz, Vellum, and Vydate), and two soil amendments (composted poultry and dairy manure). Utilizing real yield data from three field trials, we generated 500 simulations of net returns per treatment. Critically, the biological controls were the only treatments that did not predict significantly higher economic returns than non-treated fields. These results underscore the importance of improving biological control efficacy and reducing costs for these programs to be a viable option for growers. A better understanding of the antagonists' biology, including host range and virulence may increase efficacy and returns. One potentially

potent nematode biological control is the endospore-forming bacterium *Pasteuria*. The success of *Pasteuria* as a biological control requires spore attachment to the cuticle, sporulation and reproduction within the nematode host. Defining host ranges and tracking *Pasteuria* development through its lifecycle is then critical in demonstrating efficacy as a biocontrol. To understand sub-species level variation in host range, we exposed two species of root-knot nematode to a homogenous spore line of *P. penetrans* and followed parasitic success of the bacterium with 16S genotyping. Not only did we demonstrate *P. penetrans* host-species level specificity but identified other potential bacterial associates that may play a role in this interaction. To track *Pasteuria* development through its life cycle we identified homologs of sporulation regulators from the model bacterium, *B. subtilis*, in *P. penetrans*, and characterized the temporal expression of these genes. These expression markers can be used to track the process of sporulation in the nematode and augment microscopic observations to better define the efficacy of *Pasteuria*. Informed management regimes based on specific nematode populations and targeted biocontrol availability may be able to increase yields and compensate for the costs of biocontrol application. More importantly, an increase in our basic understanding of biological control activity, efficacy and plasticity may increase the practicality of biological controls through more informed management practices.

ASSESSING THE BIOGEOGRAPHY OF ENTOMOPATHOGENIC NEMATODES USING METABARCODING. **Alexandros Dritsou**las<sup>1</sup>, **Raquel Campos-Herrera<sup>2</sup> & Larry W. Duncan<sup>1</sup>.** <sup>1</sup>Citrus Research and Education Center (CREC), University of Florida (UF), 700 Experiment Station Road, FL 33850, USA. <sup>2</sup>Instituto de Ciencias de la Vid y del Vino (CSIC-Universidad de La Rioja-Gobierno de La Rioja), Finca La Grajera, Ctra. de Burgos Km. 6, 26007, Logroño, Spain.

Entomopathogenic nematodes (EPNs) are widely distributed in soils across all continents except Antarctica. Assessing the EPN community structure in an ecoregion can help reveal their biological control potential against important crop pests. Common methods for detecting EPNs in soil samples include baiting with sentinel insects, direct observation of extracted nematodes, or use of species-specific primer-probe combinations using qPCR. Less well studied is the use of metabarcoding, which has tremendous potential to characterize soil communities of EPNs and natural enemies of EPNs at lower cost and greater accuracy (because species specific tools are not required) than use of qPCR. We compared qPCR and metabarcoding to characterize food webs in soil samples from two ecoregions in Portugal. The frequency and abundance of 10 EPN species from 50 orchard and natural area sites and 13 organisms associated with EPNs were evaluated using qPCR tools, and results were published in 2018. We applied a metabarcoding approach to analyze frozen DNA samples from that study. Universal primers targeting ITS1 were used for nematode detection. All EPN species detected by qPCR were also detected by metabarcoding. The EPN species and nearly all free-living nematodes detected by both processes were highly correlated. Steinernema feltiae, the dominant EPN species, was detected by metabarcoding in 55% more sites than by qPCR. Metabarcoding also detected more EPN species than did qPCR. Sample accuracy, measured by the fit of Taylor's Power Law to data from each method, was significantly better using metabarcoding (r2=0.95) than qPCR (r2=0.76). Analysis of variance of EPN abundance in different habitats, while not identical for both data sets, resulted in the same conclusions. Canonical correspondence analysis also revealed the same abiotic variables (pH, and clay content) as most related to the community variation in both data sets. Our results suggest that metabarcoding may provide the most cost-effective and accurate means of assessing soil food webs of methods currently available.

# EFFECT OF *MELOIDOGYNE INCOGNITA* POPULATION DENSITY ON THE PREVALENCE OF *FUSARIUM OXYSPORUM* F. SP. *VASINFECTUM* RACES. **Dyer, David R.** and **K. S. Lawrence**. Auburn University, 209 Rouse Life Science Building, Auburn University, AL 36849.

Fusarium oxysporum f. sp. vasinfectum (FOV) is a fungal pathogen that causes fusarium wilt on cotton around the world. Since the discovery of the pathogen in 1892, many different biotypes have been found that cause symptoms of wilting, stunting, chlorosis and necrosis of leaves, and vascular discoloration. Some biotypes (races) of the pathogen, have been proven to have a disease interaction with some nematode species including Meloidogyne incognita, the southern root-knot nematode, while other races can devastate cotton fields without the presence of the nematode. The overall objective of the study was to evaluate the presence of FOV races in a single cotton field throughout the cotton growing season and document the effects of *M. incognita* population density and resistance traits of the cotton. The test was established in a cotton field known to be infested with a diverse history of FOV races and has sustained populations of M. incognita. Eight cotton varieties of Upland, Pima, and Acala cotton that ranged from highly susceptible to FOV and M. incognita to highly resistant to both pathogens were planted to judge the ability of these resistance traits to protect cotton form FOV infection. Symptomatic plants were collected from the field on a weekly basis for the first 6 weeks of the season and bi-weekly for the remainder of the season. FOV isolates collected were identified to race by sequencing portions of the Translation elongation factor, Beta-tubulin, and Phosphate: H<sup>+</sup> symporter genes for comparison sequences of each gene taken from references isolates. Final nematode egg data were collected at the end of the growing season. A total of 132 isolates of FOV were collected throughout the season which represented 7 different races. The most prominent race of FOV collected from the field was race 1, a race known to have a strong interaction with M. incognita. The highest nematode population density was recorded on the two Pima cotton varieties included in the test, Phytogen 800 and Pima S7 (2069 and 1539 eggs/g of root respectively). However, low rates of FOV infection, 8% on Phytogen 800 and 4% on Pima S7, were recorded on these varieties which may be due to resistance traits to FOV that are possessed by these varieties. The highest infection rate of FOV (16%) was recorded on Rowden variety of upland cotton which also supported the highest M. incognita population density (316 eggs/g of root) of any upland cotton varieties. A similar rate of FOV infection (14%) was recorded on the upland cotton variety of DP 1558NR B2XF. This is a M. incognita resistant cotton variety that supported a low population density of nematodes (76 eggs/g of root); however, the variety is also susceptible to FOV. This demonstrates that nematode resistance alone is not sufficient to protect against FOV.

NEMATOLOGY 101 – A PROPOSAL TO DEVELOP A CORE COLLECTION OF LECTURES IN PLANT NEMATOLOGY FOR THE ADVANCEMENT OF THE SCIENCE. **Eisenback, J.D.** Department of Plant Pathology, School of Plant and Environmental Sciences, Virginia Tech, Blacksburg, VA 24061.

Resources for teaching nematology are severely lacking because the subject is taught in relatively few institutions to moderately few students. As a result, the effort that is required to develop these resources cannot be easily justified.

However, if everyone who teaches nematology could be brought together to build a collection of core lectures, the quality of instruction could be improved significantly. The education committee of the Society of Nematologists has identified 12 basic areas in nematology that can be divided into more than 100 chapters that they propose to be made available three ways.First, for the nematology course with a live professor, PowerPoint lessons can be used as a backbone of the lectures presented in a course. The presentations could be used as is, or material could be added, modified, or skipped to fit the needs of the individual class. Second, for students who are interested in a particular subject, but without access to classes in nematology, the PowerPoint presentations will be recorded as narrated videos that will be made available on the internet. An individual student will be able to freely access either the individual lessons or the complete set. And thirdly, each presentation will be expanded into a chapter for a book that has all of the advantages of a textbook with additional information which contains all of the illustrations from the lessons with resources that are not available in the video, such as a list of the learning objectives, study guides and outlines, references to primary literature, suggestions for further reading, and sample questions, exams, and other resources as available.

## PROJECT NEMATODA: A COLLECTION OF EVERY SPECIES DESCRIPTION OF EVERY NEMATODE. **Eisenback, J.D**. Department of Plant Pathology, School of Plant and Environmental Sciences, Virginia Tech, Blacksburg, VA 24061.

This project was funded by the Society of Nematologists' inaugural competition for capacity building grant proposals. The purpose of Project Nematoda is to collect every species description of every nematode species that has been described since Carl Linneaus' description of *Ascaris lumbricoides* and *A. vermicularis*. The software and cloud storage package "Zotero," was selected for entering the bibliographic information into a large database. The bibliography is connected to the original species description. Thus far, the initial collection is complete for the descriptions that were in the personal collections of J.N. Sasser, H. Hirschmann, A.C. Triantaphyllou, and J.D. Eisenback. Numerous additional descriptions. Since the collection plans on entering every new species name, whether accepted or not, it is difficult to assess the percentage of names that have been already added to the database.Of the 25 thousand plus accepted species, the database contains around 50%. Some genera are more complete than others. Additions to the database will be entered as new species descriptions are collected. Nematologists who are interested in the Project are invited to join and enter additional descriptions into the database and to use it for their taxonomic research. The hope is that all nematode taxonomists will become aware of Project Nematoda and that they will enter the descriptions of missing species, as well as new descriptions, as they are published.

# DECLINE OF SUGAR MAPLES ON THE VIRGINIA TECH CAMPUS ASSOCIATED WITH DAGGER NEMATODE, *XIPHINEMA AMERICANUM*. Eisenback, J.D., and G. C. Griffin. Department of Plant Pathology, School of Plant and Environmental Sciences, Virginia Tech, Blacksburg, VA 24061.

Sugar maples (*Acer saccharum* Marshall) on the campus of Virginia Tech have been declining over the past 30 years. During the summer of 2018, eight trees with typical symptoms of dieback in the central area of the tree crown were surveyed by collecting a single sample of 250ml of soil near the main trunk in an area free from other plants, at three different times. The nematodes were extracted with a N.C. State model semi-automatic elutriator using normal operating procedures. The nematodes from each sample were counted and recorded for each of the three replicates. All eight trees had a population of dagger nematode, *Xiphinema americanum*, parasitizing the roots. Averages of the three replicates of the eight trees ranged from 20 to 346 per 250 ml, with an overall average of 131 dagger nematodes. In addition, two of the trees had moderate populations of root-knot nematodes, *Meloidogyne* spp., that are currently being increased on tomato so that they can be identified. In addition to the damage caused by dagger nematodes, several other factors may be involved in planting sugar maples in the landscape since sugar maples in natural areas surrounding the campus do not appear to be declining, but have yet to be sampled for dagger nematodes.

## INFLUENCE OF PHYSICAL AND BIOLOGICAL DISTURBANCES ON *MELOIDOGYNE INCOGNITA* RESISTANCE IN GREEN-HOUSE TOMATO PRODUCTION. **Eshchanov, Bahodir<sup>1</sup> and George Bird<sup>2</sup>**. <sup>1</sup>Tashkent State Agrarian University, Tashkent, Uzbekistan, <sup>2</sup>Michigan State University, East Lansing, Michigan, USA.

Tomatoes (*Solanum lycopersicum* L.) are commonly grown under greenhouse conditions. Grafting and pruning are used in tomato production and *Meloidogyne incognita* can be a key limiting factor. *M. incognita* control options include the use of tomato cultivars possessing the Mi 1.2 gene for resistance to *M. incognita*. Inhibition of the function of the Mi 1.2 gene can occur at soil temperatures greater than 28C. The objectives of this research were to determine the impacts of grafting, pruning (physical disturbances) and high initial population densities of *M. incognita* (biological disturbance) on *M. incognita* female development and root gall formation associated with *S. lycopersicum* cultivar Anahu after 125 to 135 days under greenhouse conditions. Anahu contains the Mi 1.2 for resistance to *M. incognita* and is a common parent for other cultivars possessing this gene. The grafting treatments consisted of non- pruned, lightly pruned and heavily pruned plants. *M. incognita* was evaluated at six initial population densities ranging from 0 to 10,000 eggs per experimental unit. In the absence of grafting and pruning, final *M. incognita* female population densities associated with low initial population densities were significantly lower on Anahu, compared to Rutgers. In the absence of pruning, there were significantly fewer root galls on non-grafted and homo-grafted Anahu, compared to Rutgers and hetero-grafting. With light pruning, at initial population densities of 1,000 and 5,000 per experimental unit, final *M. incognita*, the physical disturbances of hetero-grafting and pruning had negative impacts on the efficacy of the Anahu Mi 1.2 gene under greenhouse tomato growing conditions. TOXICITY OF TIOXAZAFEN TO *MELOIDOGYNE INCOGNITA*. **Travis R. Faske, K. Brown, and M. Emerson.** University of Arkansas-Division of Agriculture, Department of Plant Pathology, Lonoke Extension Center, Lonoke, AR 72086.

Tioxazafen (NemaStrike<sup>TM</sup> ST) is being marketed as a seed-applied nematicide in corn, cotton and soybean. Currently, there is no information on the toxicity of tioxazafen to *Meloidogyne incognita*. Second-stage juveniles were exposed to four log-dilutions of agricultural grade tioxazafen.Partial paralysis, slowed body movement, of 24-hr-old J2 was observed after 24 hr exposure to 2.70  $\mu$ g/ml tioxazafen, whereas complete paralysis was observed at 27.0  $\mu$ g/ml tioxazafen. Based on an assay of nematode motility, the 24 hr EC<sub>50</sub> value of 57.69  $\mu$ g/ml was calculated for *M. incognita*. There was no recovery in nematode motility when J2 were washed after a 24 hr exposure to a 48 hr EC<sub>50</sub> concentration of tioxazafen in a single experiment. These data provide some information on the toxicity of tioxazafen to *M. incognita*.

PLANT DEFENSE PATHWAYS MEDIATE PLANT-INSECT-NEMATODE INTERACTIONS. Filgueiras, C.C.<sup>1</sup>, L.W. Duncan<sup>2</sup>, D.W. Dickson<sup>2</sup> and D.S. Willett<sup>1</sup>. <sup>1</sup>Department of Entomology, Cornell AgriTech, Cornell University. <sup>2</sup>Department of Entomology and Nematology, CREC, University of Florida.

Entomopathogenic nematodes rely on diverse and ephemeral volatile cuestolocateandinfecttheirinsect hosts. Many of these volatile cues are released by plants. Here, we explore the role of plant defense pathways in mediating the interactions between plants, insect herbivores, and entomopathogenic nematode natural enemies. Specifically, we discuss the role of the salicylic acid pathway in linking aboveground-belowground interactions and show that stimulation of this pathway results in release of volatile cues attractive to entomopathogenic nematodes. Understanding the means by which plant defense pathways mediate the tritrophic interactions between plants, insects, and entomopathogenic nematodes not only informs our understanding of the complex belowground environment, but also suggests avenues for enhancing applied pest control with belowground natural enemies.

DEVELOPMENT OF PLANT-PARASITIC NEMATODE POPULATIONS UNDER HIGHBUSH BLUEBERRY RECEIVING VARIABLE NITROGEN INPUTS IN BRITISH COLUMBIA. Forge, Thomas<sup>1</sup>, D. Ehret<sup>2</sup>, B. Frey<sup>2</sup>, and A. Messiga<sup>2</sup>. <sup>1</sup>Agriculture and Agri-Food Canada, Summerland Research and Development Centre, Summerland, British Columbia V0H 1Z0, Canada. <sup>2</sup>Agriculture and Agri-Food Canada, Agassiz Research and Development Centre, Agassiz, British Columbia V0M 1A2, Canada, \*Retired.

Highbush blueberry is an economically important crop in British Columbia and the Pacific Northwest of the United States, and several groups of plant-parasitic nematodes have been associated with highbush blueberry in the region. Commercial highbush blueberry production in the region depends on relatively high nitrogen (N) fertilizer inputs to optimize yields, but little is known of potential non-target effects of N inputs on blueberry pests and diseases. This study examined the effects of N fertilizer rates on population development of Rotylenchus robustus, Paratrichodorus renifer and Pratylenchus crenatus in relation to blueberry production over nine years in a field experiment at Agassiz, British Columbia. The experiment was planted in fall of 2008 with six replicate plots of each of four N fertilizer (urea) rates: a non-fertilized control and N fertilizer rates of 100 (Low-N), 150 (Medium-N) and 200 (High-N) percent of N fertilizer rates recommended for growers in British Columbia, with absolute rates escalating with age of the planting. Plant-parasitic nematode populations were quantified in September of each year from 2009 through 2014, and in April, June, and September of the 2016 and 2017 growing seasons. Free-living nematode community structure was assessed at the three sample dates in 2017. Through 2014, R. robustus and P. renifer were recovered at low population densities from a low proportion of plots at the site. In 2015, population densities of R. robustus increased to levels in excess of 1500 nematodes L-1 soil where they remained through 2017. Frequency of occurrence and population densities of P. renifer also increased from 2015 through 2017, but not as extensively as R. robustus. The frequency of occurrence of P. crenatus increased from about 60% of plots at the beginning of the experiment to nearly 100% of plots through 2016 and 2017, but population densities remained modest, exceeding 250 nematodes L<sup>1</sup> soil at the 2015 sample date only. Rotylenchus robustus population densities were greater in the Low-N treatment than in the non-fertilized control and High-N treatment at three of the six sample dates in 2016 and 2017. Similarly, population densities of P. crenatus were greater in the Medium-N treatment than in the non-fertilized control and Low-N treatment at three of the six sample dates in 2016 and 2017. The nematode Structure Index and Shannon-Weiner and Simpson's indices of diversity decreased linearly with N rate. There was a significant negative correlation between population densities of R. robustus and cumulative blueberry yields, indicating the potential impact of this nematode on blueberry production under field conditions.

DISTRIBUTION AND POTENTIAL IMPACTS OF PLANT-PARASITIC NEMATODES IN VINEYARDS IN BRITISH COLUMBIA. Forge, Thomas<sup>1</sup>, P. Munro<sup>1</sup>, I. Zasada<sup>2</sup>, A. Midwood<sup>3</sup>, L. Phillips<sup>4</sup>, K. Hannam<sup>1</sup>, and M. Jones<sup>3</sup>. <sup>1</sup>Agriculture and Agri-Food Canada, Summerland Research and Development Centre, Summerland, British Columbia V0H 1Z0, Canada. <sup>2</sup>USDA-ARS, Horticultural Crops Research Laboratory, Corvallis, OR 97331. <sup>3</sup>University of British Columbia, Okanagan campus, Kelowna, British Columbia V1V 1V7. <sup>4</sup>Agriculture and Agri-Food Canada, Harrow Research and Development Centre, Harrow, Ontario N0R 1G0, Canada.

Production of winegrape (*Vitis vinifera*) has increased rapidly over the past 20 years in the Okanagan Valley, British Columbia. Several groups of plant-parasitic nematodes known or suspected to parasitize grapevines were known to exist in the region, but the extent of their distributions among vineyards had not been assessed systematically. Therefore, a survey was initiated in 2018 to examine the distribution and potential impacts of nematode species parasitizing grapevines in the region. Nematodes were extracted, identified and counted from a total of 57 vineyard blocks representing the five major soil types and latitudes used for winegrape production in the region. Complementary analyses of indicators of soil microbial biomass and soil physico-chemical properties were obtained from 25 of the vineyard samples. Ring nematodes (*Mesocriconema spp.*) were found in 82% of the vineyard blocks sampled; 81% of the blocks had root-lesion nematodes (*Pratylenchus spp.*), 77% had dagger nematodes (*Xiphinema spp.*), 25% had northern root-knot nematodes (*Meloidogyne hapla*), and 23% had stubby root nematodes (*Paratrichodorus spp.*). Prior research indicates that ring nematodes can cause significant damage to grapevines at high population densities, with 1000 ring nematodes per L soil considered to be an approximate damage threshold for newly planted vines. The overall mean ring nematode population density in Okanagan vineyards in 2018 was 1030 per L soil, with 28% of

blocks harbouring ring nematode population densities greater than 1000 per L soil. The abundance of ring nematodes was correlated with soil sand content and concentrations of extractable Cu and Zn but no other soil properties. The most widespread species of root-lesion nematodes in the region are *P. penetrans* and *P. neglectus*. Follow-up greenhouse experiments indicate that *V. vinifera* is not a host for either of these species, and we speculate that they persist in vineyard soils in the region by feeding on weeds and cover crops. While dagger nematodes were widespread, they were rarely at population densities considered damaging for *X. americanum*- group species. Taxonomic analyses of selected populations of each nematode group are ongoing. Considering the widespread distribution of ring nematodes in the region, and their potential impacts on *V. vinifera* grapevines, we conclude that they should be a primary focus of future research on impacts and management of plant-parasitic nematodes in vineyards in the region.

## SCREENING PUTTING GREEN TURFGRASS FOR STING NEMATODE FEEDING TOLERANCE **Galle, Glenn H., and J. P. Kerns**. Department of Entomology and Plant Pathology, North Carolina State University, Raleigh, NC.

The golf course industry in North Carolina is in the midst of a major transition as many courses throughout the eastern and central regions of the state are switching putting green turfgrass species from creeping bentgrass (*Agrostis stolonifera*) to bermudagrass (*Cynodon dactylon*). This change has multiple implications for management, including nematode management. Many courses throughout the state already have nematode parasitism issues, but there is very little research on nematode feeding tolerance of putting green turfgrass cultivars. The purpose of this project was to determine nematode feeding tolerance of bermudagrass and creeping bentgrass cultivars. Five cultivars of both bermudagrass and creeping bentgrass were chosen, with newer cultivars being emphasized. The study was performed in a 39 growth chamber using small conetainers of turfgrass. Plants were inoculated with sting (*Belonolaimus longicaudatus*) nematode at rates from 0-40 nematodes per conetainer. To simulate drought stress, a dry down period was initiated at 5 weeks post-inoculation. This was to determine plant stress tolerance as a result of nematode feeding. Results showed that newer cultivars of creeping bentgrass were more tolerant of nematode feeding damage than the older cultivars. A significant decrease in turfgrass quality was not observed for Pure Select or Pure Distinction until 3 days after the dry- down period was initiated. MiniVerde had the highest overall turf quality of bermudagrass cultivars. When comparing the two species, creeping bentgrass was able to sustain a higher turf quality for longer than bermudagrass with similar levels of nematode feeding pressure. Overall, the results indicate that while the newer cultivars may not be specifically screened for nematode feeding tolerance, the improved plant characteristics help these cultivars tolerate nematode feeding damage to roots.

EFFECT OF NEW BIOLOGICALLY-ACTIVE NEMATICIDES AS POST-PLANT APPLICATIONS FOR MANAGEMENT OF RE-PLANT DISEASE CAUSED BY *MESOCRICONEMA ORNATUM* UNDER FIELD CONDITIONS Jagdale<sup>1</sup> Ganpati B., P. M. Brannen<sup>1</sup>, J. Oliver<sup>2</sup>, R. Allen<sup>3</sup> S. Curry<sup>4</sup>, W. Lovett<sup>5</sup> M. Frye<sup>6</sup>, J. Slusher<sup>7</sup>, J. Shealey<sup>8</sup>, T. Holladay<sup>1</sup>, P. Severns<sup>1</sup>, and D. Shapiro-Ilan<sup>9</sup> <sup>1</sup>Dept. of Plant Pathology, University of Georgia, Athens, GA, 30602. <sup>2</sup>Dept. of Plant Pathology, University of Georgia, CAES Campus, Tifton, GA 31794. <sup>3</sup>University of Georgia Extension, Alma GA 31510. <sup>4</sup>University of Georgia, Appling County Extension, Baxley GA 31515. <sup>5</sup>University of Georgia, Bacon County Extension, Alma GA 31510. <sup>6</sup>University of Georgia, Wayne County Extension, Jesup GA 31598. <sup>7</sup>University of Georgia, Ware County Extension, Waycross, GA 31503. <sup>8</sup>University of Georgia, Echols County Extension, Statenville, GA 31648. <sup>9</sup>US-DA-ARS Southeastern Fruit & Tree Nut Res. Lab. 21 Dunbar Road, Byron, GA 31008.

Blueberry replant disease is an emerging threat to continued blueberry (Vaccinium spp.) production in Georgia (GA). Blueberry replant disease symptoms present in the field as uneven and/or stunted growth of plants, and replant disease is associated in GA with high populations of the ring nematode (Mesocriconema ornatum). We initiated four field experiments on May 8, 2018 in established blueberry farms located in four different Georgia counties including Appling, Bacon, Ware and Wayne to compare the efficacy of new organic nematicides (Nortica 10 WP, Majestene, MeloCon WG, metabolites of two entomopathogenic bacteria (Xenorhabdus szentirmaii and X. bovienii) and an organic amendment (pine bark) with a conventional chemical nematicide (Velum Prime; fluopyram) against naturally occurring M. ornatum 30 and 180 days after treatment (DAT). We found that in Appling County the application of all three new organic compounds, two bacterial metabolites, a chemical compound and pine bark compost did not significantly reduce the population density of blueberry ring nematodes compared to the untreated control (water) under field conditions at 30 and 180 DAT. However, the application of pine bark, metabolites of X. szentirmaii and X. bovienii bacteria, and fluopyram significantly reduced the reproduction of ring nematodes compared to the untreated control (water) 180 DAT. However, ring nematode multiplication rates were not similarly reduced by the same treatments in Bacon, Ware, and Wayne counties. It is possible that the inconsistent treatment responses between sites is due to a true lack of treatment efficacy or that the initial nematode concentrations varied widely enough to mask the treatment effects measured by percent growth. Although we observed lowered reproductive rates of ring nematodes after application of pine bark, entomopathogenic bacterial metabolites and fluopyram at 180 DAT in the Appling County experiment, further experimentation under tightly controlled conditions will be necessary to confidently ascribe the effects of different treatments on ring nematode growth rates.

DIFFERENTIAL MORPHOGENETIC CYST CHARACTERISTICS OF *HETERODERA* SPECIES. Garam, Han<sup>1</sup>, H. I. Kang<sup>2</sup>, I. S. Choi<sup>2</sup> and Y. H. Kim<sup>1</sup>. <sup>1</sup>Department of Agricultural Biotechnology and Research Institute of Agriculture and Life Sciences, Seoul National University, Seoul 08826, Republic of Korea. <sup>2</sup>Department of Plant Bioscience, Pusan National University, Miryang 50463, Republic of Korea.

Presently there are two morphologically similar soybean cyst nematodes, *Heterodera glycines* and *H. sojae* in Korea. This study aimed to examine morphogenetic characteristics of cysts in the two cyst nematodes and sometimes *H. trifolii* found in Chinese cabbage to develop microscopic techniques for the diagnosis of the two soybean cyst nematodes. The cyst sizes {length (L) and widest width (W)} were significantly different in the order of *H. trifolii*, *H. glycines*, and *H. sojae*. There were no significant differences in the rates of L/W among the three *Heterodera* species. The cyst body walls of *H. sojae* were significantly thicker than those of *H. glycines* and *H. trifolii*. There were no significant differences in fecundity of the cyst; however, the hatching rates of *H. sojae* in water were extremely low, compared to *H. glycines*, suggesting *H. sojae* may be more persistent in fallowing fields than *H. glycines* because of the total low emergence rates without host plants

and moreover thickened cyst body wall of *H. sojae*. The slopes of the vulval cone were more rapid  $(50.6^{\circ})$  in *H. glycines* with its vulval cone surfaces of high and steep striations than *H. sojae*  $(102.2^{\circ})$  with those of low and smooth striations. All of these results may provide information useful for the diagnosis of *H. glycines* and *H. sojae* from soybean fields, especially for cyst sizes and slopes of vulval cones that can be examined using a simple stereomicroscope.

# POTENTIAL MECHANISM OF ACTION FOR *BACILLUS* SPP. INDUCING RESISTANCE TO *MELOIDOGYNE INCOGNITA* ON COTTON. Gattoni, Kaitlin, Xiang, N., Lawaju, B., Lawrence, K.S., Park, S.W., and Kloepper, J.W. Department of Entomology and Plant Pathology, Auburn University, Auburn, AL, 36849.

Meloidogyne incognita, the southern root-knot nematode, is an economically damaging plant-parasitic nematode that affects many crops. One management strategy is to use biological control agents, which can work by direct antagonism or indirect antagonism that encompasses induced systemic resistance and systemic acquired resistance. The objective of this study is to determine the mechanism of action of 5 Bacillus spp. used to manage M. incognita. Three species, B. mojavensis strain 3, B. velenzensis strain 2, and B. pumilus GB34, were obtained from Dr. Kloepper's lab at Auburn University. Two species, B. amyloliquefaciens QST713 and B. firmus I-1582, are the active ingredients of Bayer's Serenade and VOTiVO, respectively. In this study, a greenhouse assay was used to determine the efficacy of the Bacillus spp. An in vitro assay determined any direct antagonistic capabilities of the bacteria. A split root assay and RT-qPCR determined the systemic capabilities. The final assay, a qPCR, determined how well the Bacillus spp. could colonize the cotton roots. Data were statistically analyzed in SAS 9.4 using the Glimmix procedure. In the greenhouse assay, the chemical standard of fluopyram decreased nematode population density by 99% compared to the untreated control (P≤0.05). Bacillus firmus I-1582 and B. amyloliquefaciens QST713 also significantly decreased nematode population density by 75% and 79%, respectively ( $P \le 0.05$ ). During the *in vitro* assay, only *B. firmus* I-1582 increased percent mortality compared to the water control from 6% mortality to 78% mortality (P≤0.05). Three Bacillus spp. displayed systemic capabilities in the split root assay. Two species, B. amyloliquefaciens QST713 and B. firmus I-1582 significantly decreased the nematode population density when on the same root half as the nematode, by 68% and 78% respectively, and when inoculated on the root half opposite the nematode, by 86% and 84% respectively, compared to the nematode control ( $P \le 0.1$ ). Bacillus mojavensis strain 3 significantly decreased nematode population density when inoculated on the root half opposite the nematode by 82%, but did not decrease numbers when inoculated together on the same side. In the RT-qPCR, GhOPR3 and GhLOX1, two genes correlating to jasmonic acid (JA), and  $\beta$ -1,3-glucanase, a gene correlating to salicylic acid (SA), were observed at 4 different time points after inoculation with *B*. amyloliquefaciens QST713, B. firmus I-1582 and M. incognita second stage juveniles. At 24 hours, the Bacillus spp. upregulated GhLOX1 but not GhOPR3, leading to the conclusion that a JA-independent intermediate molecule, most likely OPDA, is involved in the short-term systemic resistance conferred by the bacteria. At 1 week,  $\beta$ -1,3-glucanase was upregulated by both *Bacillus* spp., indicating a long-term SA-dependent systemic response. The final qPCR determined these two Bacillus spp. were able to successfully colonize the plant roots for at least 24 days. These results will help to implement these species into an integrated pest management strategy.

PLANT PARASITIC NEMATODES ASSOCIATED WITH GRAPEVINES, VITIS VINIFERA, IN NEW MEXICO. **Giese, Gill<sup>1</sup>, J.M. Beacham<sup>2</sup>, C. Velasco-Cruz<sup>1</sup>, S. Thomas<sup>2</sup>, and T.O. Powers<sup>3</sup>**. <sup>1</sup>Department of Extension Plant Sciences, P.O. Box 30003, MSC 3AE, New Mexico State University, Las Cruces, NM 88003. <sup>2</sup>Department of Entomology, Plant Pathology and Weed Science, P.O. 30003 MSC 3BE, New Mexico State University, Las Cruces, NM 88003. <sup>3</sup>Department of Plant Pathology, 406 Plant Science, University of Nebraska-Lincoln, Lincoln NE 68583.

A survey of twenty-five commercial vineyards from three American Viticultural Areas (AVAs) within New Mexico, USA was completed in 2018 to determine the association of plant-parasitic nematodes with vineyards and vine health and to investigate possible correlations with measured soil analysis parameters. The most commonly encountered plant-parasitic nematodes in New Mexico were Meloidogyne spp., and Xiphinema americanum (detected in >50% of sampled vineyards) with Pratylenchus spp., Helicotylenchus spp., and Mesocriconema spp. also detected. The frequency of nematode occurrence exceeded levels reported to cause moderate to extensive damage to producing vineyards in California (UC Agriculture and Natural Resources Publication 3343, third ed.). Highest populations of Meloidogyne and Pratylenchus were 97-fold and 21-fold greater, respectively, than those associated with extensive damage in California. Highest populations of Xiphinema americanum and Mesocriconema were 13-fold and 11-fold greater, respectively, than populations associated with moderate damage to producing grapevines in California. Correlation analyses indicate that *Meloidogyne* spp. populations are not impacted by the presence of other nematodes or soil parameters. Plant parasitic nematodes other than Meloidogyne spp, were positively correlated with soil parameters related to vine growth and health, specifically soil pH and boron. Numbers of Xiphinema americanum, Mesocriconema, and Pratylenchus spp were positively correlated. Mononchid numbers were strongly correlated to these three ectoparasitic nematodes and negatively correlated to the bacterivore population. DNA barcoding with the COI mitochondrial gene is underway for better taxonomic resolution of the Pratylenchus, Meloidogyne and Mesocriconema species. To our knowledge such a survey has not been previously accomplished, and the long-term impact of these nematodes on New Mexico vineyards, especially own-rooted V. vinifera vineyards, has not been determined and merits further study.

## EVALUATION OF NIMITZ<sup>®</sup> NEMATICIDE IN COMBINATION WITH LABELED FUNGICIDES TO DEVELOP A NON-FUMI-GANT PROGRAM FOR FRUITING VEGETABLES. P. A. Navia Gine and D.J. Erasmus. ADAMA Agricultural Solutions, Raleigh, North Carolina.

NIMITZ (Fluensulfone 480EC) is an efficacious nematicide providing control of plant-parasitic nematodes with minimal impact on beneficial nematodes. In comparison to fumigants, application of NIMITZ is simple, and "… represents a safer alternative for nematode control with a new mode of action and a much simpler and straightforward product label." (U.S. Environmental Protection Agency, Federal Docket, July 24, 2014). NIMITZ is labelled with a 'CAUTION' signal word. The product requires no Fumigant Management Plans, no

24-hour field monitoring, no buffer zones, has a 12 hr re- entry interval (REI) and minimal personal protective equipment (PPE). Traditionally, fumigants have been used to control and suppress soil-borne diseases and nematodes in the early growth stage of tomatoes (Solanum lycopersicum) and peppers (Capsicum annuum) produced under plastic mulch. However, the ban on methyl bromide coupled with increasingly stringent fumigant regulations has increased the interest in non-fumigant alternatives for nematode control (Morris K., 2015) and soil-borne diseases. With the development and registration of NIMITZ and other new 3 Fluorine non-fumigant true nematicides (Desaeger J., 2017), new tools are available to develop a non-fumigant alternative for several production systems. Increasing regulatory and consumer pressure is driving the use of softer chemistries in production. With the objective of screening different non-fumigant fungicides to be used in combination with NIMITZ, 6 trials in tomatoes and peppers at different locations in Florida where conducted in 2017 and 2018 in an ongoing development effort. NIMITZ at labeled rates was applied with a 7-day pre-plant broadcast spray and then incorporated mechanically. Selected treatments included tank mixes with fungicides labeled for tomatoes and/or peppers and NIMITZ . After the broadcast application, a standard fungicide program with drench and/or drip injected applications at planting and after planting were applied according to The University of Florida (UF/IFAS) and The University of Georgia (UGA) extension recommendations. Preliminary results show positive indications: 1) Good protection of the roots, especially from nematode infections in the early establishment phase of the crop, provided less incidence of secondary infections caused by soil-borne diseases; 2) A good follow-up fungicide program to address fungi classes like oomycetes, ascomycetes and others as needed, provided suppression of fungi infections; and 3) Yield evaluations showed that there were minimal significant differences compared with the fumigated treatments. While the data are not conclusive, studies will be continued to validate preliminary observations.

INFLUENCE OF *PARATYLENCHUS PROJECTUS* ON THE GROWTH OF NINE VEGETABLES. **Giyoon, Kwon<sup>1</sup>, H. Kang<sup>1</sup>, J. Seo<sup>1</sup>, N. Park<sup>2</sup>, E. Yun<sup>2</sup>, D. Kim<sup>2</sup>and I. Choi<sup>1,2</sup>**.<sup>1</sup>Pusan National University, Miryang 50463, Korea. <sup>2</sup>Nematode Research Center, Pusan National University, Miryang 50463, Korea.

In 2018, organic lettuce grown in plastic house was found severely damaged by *Paratylenchus projectus* in Korea. The nematode caused almost total yield loss on marketable lettuce. In responds of farmers demands, we selected nine market-favored vegetables and tested for possible rotation crops with lettuce. The nine vegetables were: *Brassica juncea*, *B. oleracea* var. *acephala*, *B. oleracea* var. *gonglodes*, *B. oleracea* var. *italica*, *B. rapa*, *B. rapa* subsp. *nipposinica*, *Cichorium endivia*, *C. intybus* and *Ipomoea aquatica*. Each vegetable seedling was planted in 10-cm clay pots and inoculated with 6 *P. projectus* nematodes per g of soil and replicated for ten times. After two months grown in greenhouse, inoculated plants reduced the plant height, number of leaves and root weight. In an average, nine vegetables reduced plant weight about 52.6% compared to non-inoculated control; yield reduction was 34.1% in *C. endivia*, 39.9% in *B. oleracea* var. *gonglodes*, 42.5% in *B. oleracea* var. *acephala*, 49.4% in *C. intybus*, 49.7% in *I. aquatica*, 54.2% in *B. rapa*, 61.2% in *B. rapa* subsp. *nipposinica*, 63.9% in *B. oleracea* var. *italica* and 78.2% in *B. juncea*.

### LOOKING BELOW THE SURFACE: DO DIFFERENT CROPPING SYSTEMS AFFECT THE NEMATODE COMMUNITY BELOW THE PLOW LAYER? **Grabau, Zane<sup>1</sup>, L.A. Schumacher<sup>1</sup>, R. Sandoval-Ruiz<sup>1</sup>, H. Liao<sup>2</sup>, I. Small<sup>2</sup>, and D.L. Wright<sup>2</sup>.** <sup>1</sup>Entomology and Nematology Dept., University of Florida, Gainesville, FL 32611. <sup>2</sup>North Florida Research and Education Center, University of Florida, Quincy, FL 32351.

Plant-parasitic nematodes are known to reside below the plow layer (approximately the top 30 cm in the soil profile) in agricultural systems. Roots of agronomic crops such as cotton can reach below the plow layer, so biological activity at this depth may affect plant productivity, but relatively little is known about non-parasitic soil microfauna, including free-living nematodes, below the plow layer. Our hypothesis was that free-living nematodes would be present below the plow layer and that rotations where crops were grown for a greater portion of the year would enhance biological activity, particularly living nematode abundances, below the plow layer. Two studies in Quincy, Florida were used to test this hypothesis. In the first study, conventional rotation (cotton-cotton-peanut) and sod-based rotation (2 years of bahiagrass-peanut-cotton) were compared in a long-term study where each phase of each rotation were present each year. In 2017 and 2018, soil was collected to 4 feet deep and using a hydraulic probe and nematode soil populations were analyzed in 30-cm sections before planting, after harvest, and during winter. In the second study, cotton, peanut, corn and soybean summer crop treatments are crossed with fallow-fallow, oats-carinata, and carinata-fallow winter crop treatments. Soil nematode populations were assessed at 0-30 and 30-120 cm deep in the soil profile after harvest in 2017 and 2018 and before planting in 2018. In both studies, abundances of free-living nematodes decreased substantially below the plow layer. Crop rotations affected nematode community composition in the plow layer. In particular, fungivores were more abundant in pasture bahiagrass than cotton or peanut. Fungivores and bacterivores were scarce below 30 cm with 33 nematodes/100 cm<sup>3</sup> across multiple seasons of the two studies, which was 7% of the 461 nematodes/100 cm<sup>3</sup> soil in the upper 30 cm. Crop rotations affected nematode community composition in the plow layer, but free-living nematode abundances below the plow layer were generally too small for crop rotation effects to be biologically meaningful. In contrast, reniform nematode abundances were greatest in the top 30 cm (3163 nematodes/100 cm<sup>3</sup> soil), but abundances were still substantial below 30 cm at 1312 nematodes/100 cm<sup>3</sup> soil or 42% of abundance in the top 30 cm. Crop rotations generally affected reniform nematodes throughout most of the soil profile. Results of these studies suggest that, across cropping systems, most biological activity of non-parasitic organisms occurs in the plow layer, even in a biennial pasture bahiagrass system. In contrast, reniform nematode activity is not restricted to the plow layer, and crop rotation practices can help manage this nematode, even deep in the soil profile.

## UNMANNED AERIAL SYSTEM IMAGERY ANALYSIS OF MODERN TURFGRASS NEMATICIDES. Groover, Will and K. Lawrence. 209 Rouse Life Science Building, Auburn University, Auburn, AL 36849.

Plant-parasitic nematodes are one of the most problematic and damaging pathogens of turfgrass in the southeastern United States, yet symptoms can often go misdiagnosed, causing nematode population density to reach critical levels before detection. With early detection being one of the most important strategies for managing plant- parasitic nematodes, implementing a scouting strategy in conjunction with soil sampling

is very important. Recently, the Federal Aviation Administration opened airspace to routine commercial drone use, causing the amount of permits and use in agricultural research to increase exponentially. Little to no research has been conducted on using this technology as a tool for nematode management. The objective of this project is to evaluate Unmanned Aerial Systems (UAS) for their ability to detect plant-parasitic nematode damage in turfgrass through the Normalized Difference Vegetation Index (NDVI) and Normalized Difference Red Edge Index (NDRE) in conjunction with nematicide applications. Both of the indexes used in this study are closely associated with plant health. In 2018, microplot trials were conducted on 'TifWay 419' hybrid bermudagrass. Individual microplots had previously been inoculated with either Meloidogyne incognita or Belonolaimus longicaudatus. Nematicides included Multiguard Protect (furfural), Nimitz Pro G (fluensulfone), Divanem (abamectin), Indemnify (fluopyram), and an untreated control as a comparison. Image analysis was collected via a DJI Phantom 4 equipped with a Micasense RedEdge-M camera at three time points: prior to nematicide treatment, 30 days after treatment (DAT), and 60 DAT. Nematode population counts per 100 cm<sup>3</sup> of soil were also taken the same time as image collection from each plot. Data were analyzed using analysis of variance (SAS 9.4), and means were compared using the Dunnett's statistic with  $P \le 0.05$ . All nematicides led to a significant reduction of *M*. *incognita* at 30 DAT, and all but abamectin led to a significant reduction of *B. longicaudatus* at 30 DAT ( $P \le 0.05$ ). At 60 DAT, fluopyram was significantly lower than the untreated control in both nematode population densities ( $P \le 0.05$ ). Abamectin had a significantly lower *M. incognita* population density compared to the untreated at 60 DAT ( $P \le 0.05$ ). All other nematicides were statistically similar to the untreated at 60 DAT  $(P \le 0.05)$ . All treatments except furfural saw a significant increase in NDVI and NDRE values compared to the untreated control at 30 DAT, indicating an increase in plant vigor ( $P \le 0.05$ ). At 60 DAT, only fluopyram had a significantly higher NDVI value compared to the untreated, and NDRE values were statistically similar across all treatments. Overall, all nematicides were effective at lowering both M. incognita and B. longicaudatus population densities, and positive trends were observed for both NDVI and NDRE indexes for plant health analysis. This data shows that Unmanned Aerial System imagery can be a valuable tool for vigor assessments related nematode population densities in turfgrass.

### METABOLITES THAT MAY EXPLAIN ANTAGONISM BETWEEN *BELONOLAIMUS LONGICAUDATUS* INFESTED BERMU-DAGRASS ROOTS AND *PYTHIUM ARRHENOMANES*. Gu, Mengyiand W. T. Crow. Entomology & Nematology Dept. University of Florida, Gainesville, FL 32611.

The fungus-like oomycete *Pythium* causes *Pythium* root rot, one of the most common bermudagrass diseases on Florida golf courses. Plant-parasitic nematodes (PPN) were observed from most *Pythium* root rot disease samples received by UF/IFAS Plant Diagnostic Center in 2017. A disease interaction experiment indicated that different PPN species and different *Pythium* spp. interact with each other differently. In lab experiments *Belonolaimus longicaudatus* infested roots consistently reduced mycelia growth of *P. arrhenomanes*. In this experiment, bermudagrass root exudates from plants inoculated with *B. longicaudatus*, *P. arrhenomanes* or noninoculated were filtered, sterilized, and freeze dried. These samples were then analyzed using a LC-MS global metabolomics test. Compounds in the samples were identified and compared among treatments. Increased levels of benzene sulfonic acid and azelaic acid were observed in bermudagrass root exudates following inoculation with *B. longicaudatus*. Benzene sulfonic acid provides an acidic environment, which may inhibit *Pythium* mycelia growth. Azelaic acid is involved in the plant immune system and may induce plant resistance to *Pythium* spp. The presence of benzene sulfonic acid and azelaic acid in PPN infested root exudates provides a possible explanation of the antagonistic effects observed between *B. longicaudatus* infested roots and *P. arrhenomanes*.

INTERACTION BETWEEN *RADOPHOLUS SIMILIS, PRATYLENCHUS COFFEAE*, AND *MELOIDOGYNE INCOGNITA* IN SEED-LINGS OF *MUSA* AAB (SUBGROUP PLANTAIN) "DOMINICO HARTÓN." **Guzmán-Piedrahita, Oscar A.<sup>1</sup>, C. Zamorano- Montañez<sup>1</sup>, and H. D. Lopez-Nicora<sup>2</sup>.** <sup>1</sup>Programa de Doctorado en Ciencias Agrarias, Universidad de Caldas, Manizales, Caldas, Colombia. <sup>2</sup>Departamento de Producción Agrícola, Universidad San Carlos, Alfredo Seiferheld 4989, Asunción, Paraguay.

Plant-parasitic nematodes attack roots and corms of musaceae plants, affecting the consumption of water and nutrients, which leads to a lower growth and crop yield. In Colombia, there is little or no information regarding the losses and damages cause by Radopholus similis, Pratylenchus coffeae, and Meloidogyne incognita in Dominico Hartón plantation plantations both in nursery and field conditions. In this research, the effect of the three-way interaction between Radopholus similis, Pratylenchus coffeae, and Meloidogyne incognita on the development of Dominico Hartón plantain seedlings was evaluated. The study was conducted in a nursery, under field conditions at the Montelindo Farm of the Universidad de Caldas, located in the town of Palestina, Colombia. Samples, however, were processed in the nematology laboratory of Universidad de Caldas. Plantain seeds (corms) were planted in bags containing sterilized soil and placed on stainless steel tables 50 cm above the ground. Forty days after planting, these seedlings were inoculated with 750, 1,500, and 3,000 R. similis alone. Similarly, using the same nematode densities, other seedlings were inoculated with P. coffeae and M. incognita alone. In addition, three interaction treatments consisted of seedlings infested concomitantly with R. similis, P. coffeae, and M. incognita at different population densities. The interaction treatments received 750, 1,500, and 3,000 nematodes of each species, respectively. Uninoculated seedlings were included as control. Twelve weeks after inoculation, plant height, above-ground dry matter and nematode reproduction factor (RF = final population [in soil and roots]/initial population [inoculation levels]) were calculated. Treatments (with ten replications each) were evaluated under a randomized complete block design. Except for the treatment that received 3,000 M. incognita alone, all nematodes alone and in combination, significantly (p<0.005) reduced plant height compared to the control. On the other hand, only M. incognita alone and in combination with R. similis and P. coffeae reduced above-ground dry matter compared to the control. All nematodes significantly (p<0.005) produced a RF greater than 1 except for P. coffeae which had a maximum RF value of 0.24. We conclude that the growth of Dominico Hartón plantain seedlings was affected by R. similis, but the greatest damage occurred in combination with P. coffeae and M. incognita.

IN VIVO SCREENING OF FUNGI FROM THE CULTURABLE SOYBEAN CYST NEMATODE CYST MYCOBIOME. **Haarith, Deepak**<sup>1</sup>, **D.G. Kim**<sup>2</sup>, **S. Chen**<sup>1</sup>, **and K.E. Bushley**<sup>3</sup>. <sup>1</sup>Plant Pathology. <sup>2</sup>Biochemistry, Molecular Biology, and Biophysics. <sup>3</sup>Plant and Microbial Biology, University of Minnesota, Saint Paul, MN, 55108. Fungi cultured from mycobiome from Soybean Cyst Nematode (SCN) cysts across a 3-year period between 2014 and 2016, were screened *in vitro* and *in vivo* for their potential biocontrol activity against SCN eggs. Previously, over 5000 fungal isolates from the SCN mycobiome were identified to genus level based on their ITS DNA barcode sequence and were clustered into 326 clusters based on 97% sequence similarity. A representative of each cluster was screened for their ability to parasitize SCN eggs and cyst and/or to inhibit SCN egg hatch *in vitro* laboratory based bioassay. We selected the top ten egg parasites and the top ten egg hatch inhibitors to test *in vivo* in growth chamber-based assays for their biocontrol potential on SCN. The screening was conducted on 5 replicate "sturdy" soybean plants in separate 6" cone-tainers, with nematode inoculum levels of 10,000 eggs/100 cc of soil and no-SCN controls. In addition to the twenty isolates inoculated at 10<sup>6</sup> spores/ 100 cc of soil, we included Poncho/VoTivo as positive control and a no-fungus control. We also tested three different soil types; autoclaved 80:20::sand:field-soil, fresh field soil, and autoclaved field soil to account for possible interactions between the test organisms and other fungi in the soil microbiome. Plant live/ dead status and height were measured at 30 and 60 days post fungal inoculation. Cysts and eggs were extracted and enumerated from each cone separately. Fungal isolates were ranked based on both the plant health parameters and the level of SCN reproduction. Several isolates identified *in vitro* as good egg parasites performed better than no-fungus control and Poncho/VoTivo. Most of the isolates identified as high hatch inhibitors did not exhibit biocontrol potential. We repeated the experiment for the three best performing isolates and three underperforming isolates using autoclaved 60:40::sand:field soil alone to check for reproducibility and evaluated performance under a lower inoculum level (3000 SCN eggs 10<sup>5</sup> fungal spores

# FIRST REPORT OF THE ROOT-KNOT NEMATODE *MELOIDOGYNE JAVANICA* INFECTING GIANT BAMBOO (*DENDROCAL-AMUS ASPER*) IN FLORIDA. Habteweld, Alemayehu, M. L. Mendes, and W. T. Crow, Nematode Assay Laboratory, Entomology and Nematology Department, University of Florida.

Plant-parasitic nematodes (PPN) are considered as economically important pests of different plant species in Florida, but knowledge on PPN, especially root-knot nematode (RKN) species, infecting bamboo is limited. Presence of pin nematode (Gracilacus latescens), bamboo cyst nematode (Afenestrata koreana), southern root-knot nematode (Meloidogyne incognita), awl nematode (Dolichodorus heterocephalus), burrowing nematode (Radophulus similis), ring nematode (Mesocriconema spp.), stubby nematode (Paratrichodorus teres), dagger nematode (Xiphinema sheri and X. coxi) were reported from different species of bamboo in Florida previously. In 2018, soil and root samples were submitted to the University of Florida Nematode Assay Lab for a routine nematode diagnosis associated with stunted giant bamboo (Dendrocalamus asper) from Oxford, FL being grown commercially for production of edible bamboo shoots. A high population density of J2 of RKN (179/100 cm<sup>3</sup> of soil) were extracted from soil using decanting and sugar centrifugal flotation technique. Several J2 (500/10g roots) were also extracted by incubating the roots in a mist chamber for 72 hours and adult females were observed infecting the bamboo roots. Molecular analysis was conducted for the proper determination of the RKN species. Genomic DNA was extracted from 5 J2 obtained from the root samples to characterize the mitochondrial haplotype. The intergenic spacer and part of the adjacent large subunit ribosomal RNA gene (lrDNA) were amplified using MORF/MTHIS and TRNAH/MHR106 primer pairs. The sequence polymorphism in lrDNA was revealed by restriction pattern following digestion with the restriction enzymes Hinfl and Mnll. MORF/MTHIS and TRNAH/MHR106 primer pairs amplified approximately 743- and 558-bp, respectively. The restriction digestion of the 558-bp obtained from TRNAH/MHR106 primer pairs produced 558-(no digestion), and 341-, 140- and 77-bp fragments for HinfI and MnII, respectively. The PCR products size (743- and 558-bp) obtained from the two primer sets and the restriction patterns were typical for M. javanica. Finally, the species identity was confirmed by javanica-specific primer set (Mi-F/Mi-R) that produced approximately 670-bp DNA fragment. The molecular identification demonstrated that the population of RKN nematodes extracted from the roots of giant bamboo was M. javanica. To our knowledge, this is the first report of the M. javanica infecting giant bamboo in Florida. The nematode isolate is being reared on tomato and will be used to verify its pathogenicity to giant bamboo.

#### EFFECT OF FLUOPYRAM AND OXAMYL AT DIFFERENT APPLICATION TIMES ON THE CEREAL CYST NEMATODE *HET*-ERODERA AVENAE UNDER GREENHOUSE CONDITIONS. **Hafez, Saad L.<sup>1</sup> and I. Soliman<sup>1</sup>**. 29603 U of I Lane, Parma, ID 83660, USA.

The Cereal Cyst Nematode (CCN), Heterodera avenae is considered an important plant parasitic nematode on wheat worldwide. H.avenae has been reported on wheat with different climatic types. It is considered an important plant parasitic nematode in the Pacific Northwest (PNW). It is estimated that this nematode reduces wheat profitability by at least \$3.4 million annually in the PNW states of Idaho, Oregon, and Washington. Aldicarb, spirotetramat and a few other nematicides were tested for controlling CCN; however, no nematicide is registered yet for this nematode in the U.S. Greenhouse experiments were conducted to evaluate the effect of Velum Prime (fluopyram) and Vydate (oxamyl) on H. avenae grown on the susceptible wheat cultivar "Alturas". Fluopyram at a rate of 6.5 oz/a was very effective at reducing the mean number of white females in both natural and artificial inoculation. In the artificial inoculation with an initial inoculum of 10 eggs/g soil, no white and brown females reproduced when fluopyram was applied either one week before inoculation or at inoculation compared with means of 26.0 and 1.8 white and brown females per 500 cc soil, respectively, in the untreated pots. Application of fluopyram after hatching (8 weeks after wheat emergence) reduced the mean number of the white females to 0.0 compared to 26.0 per 500 cc soil in the untreated pots. The same effect was observed in the naturally infested soil with 6.5 eggs+J2/g. Application of fluopyram at planting and after hatching reduced the mean number of white females to 0.6 and 5.2, respectively, compared to 27.4 per 500 cc soil in untreated pots. No females reproduced in the oxamyl application one week before inoculation (artificial inoculation) at an application rate of 4.2 pt/a. Oxamyl applied at inoculation resulted in 1.2 white and .4 brown females. When oxamyl was applied after hatching, 6.8 white and 3.6 brown females were counted. The application of oxamyl at inoculation and after hatching in naturally infected soil reduced the mean number of white females to 11.0 and 10.6, respectively, compared to 27.4 in untreated pots per 500 cc soil. Brown females were not counted in the naturally infected treatments to avoid mix-up with the main inoculum. Fluopyram and oxamyl induced the white females turn faster to brown females with fewer encysted eggs compared to untreated pots. There were few significant differences in the plant heights, top fresh and dry weights, root fresh and dry weights and grain yield in both experiments. We assume that the damage of the plant is correlated to the long field infection history of this nematode since it has only one generation per year and low hatch sizes. Ongoing research of the same treatments under outdoor conditions will be harvested and analyzed two months from the writing of this abstract.

## LIFE CYCLES OF ENTOMOPATHOGENIC NEMATODES CAPTURED ON VIDEO. Haines, Stephanie, D. Londoño, and J. Maness. BASF, Research Triangle Park, NC 27709.

By observing the infection, death and decay of host insects, we were able to capture photographs and videos of entomopathogenic nematodes developing from infective juveniles to adults and to new generations of infective juveniles. Entomopathogenic nematodes (EPNs) require species-specific hosts, therefore different nematode species produced commercially are registered for controlling specific pests. We took three different nematode species: *Steinernema feltiae*, *Steinernema carpocapsae*, and *Heterorhabditis bacteriophora* and applied them to three host insects: fungus gnats (*Bradysia spp*), codling moths (*Cydia pomonella*), and white grubs (*Phyllophaga spp*.), respectively. Nematodes and host insects were applied to agar plates (for clear visibility), soil and leaves (to simulate field conditions), kept in growth chambers, and periodically monitored through microscopes. Photograph and video quality improved as we refined methodology with selections of substrate, environmental conditions, lighting, and camera/microscope hardware and software.

# EFFECTS OF NON-FUMIGANT NEMATICIDES ON INCREASING INITIAL POPULATION DENSITIES OF *MELOIDOGYNE IN-COGNITA* ON CUCUMBER. Hajihassani, Abolfazl<sup>1</sup>, R. F. Davis<sup>2</sup>, and P. Timper<sup>2</sup>. <sup>1</sup>Department of Plant Pathology, University of Georgia, Tifton, GA 31793. <sup>2</sup>Crop Protection and Management Research Unit, USDA ARS, Tifton, GA 31793.

The southern root-knot nematode (*Meloidogyne incognita*) is the most important yield-limiting plant-parasitic nematode, affecting all vegetable crops including cucumber (*Cucumis sativus* L.). Management of RKN in cucumber production depends on chemical treatments with fumigants and non-fumigant nematicides. Understanding the relationship of varying population densities of root-knot nematodes to nematicides could lead to more efficient use of nematicides to maximize nematode control with minimum cost. Two experiments were conducted in which four nonfumigant nematicides (oxamyl at 4.67 liter a.i./ha, fluopyram at 0.50 liter a.i./ha, fluensulfone at 5.84 liter a.i./ha, and fluazaindolizine at 4.48 liter a.i./ha) were examined for their efficacy in reducing gall severity and post-harvest soil nematode counts in microplots inoculated with different initial population densities (1,000, 5,000, 10,000, and 20,000 *M. incognita*/microplot), and improving cucumber yield. Nematicides were applied one day prior to transplanting cucumber seedlings, except fluensulfone which was applied 7 days before transplanting. All four nematicides were effective (*P*<0.05) in reducing the root gall index when compared with the untreated control on a consistent basis at all *M. incognita* initial population densities. At the lowest population density, no significant difference in gall index or final population density was observed among nematicides; however, gall index increased with increasing initial population densities in nematicide-treated microplots. Correlations between gall index and initial population density clearly showed that soil treatment with fluensulfone, fluazaindolizine or fluopyram was more effective in reducing gall severity than treatment with oxamyl. Regression analysis also indicated no significant effect of nematode population densities on yield of cucumber treated with these nematicides.

IDENTIFICATION OF ROOT-KNOT NEMATODE RESISTANCE IN *SOLANUM SISYMBRIIFOLIUM*. Hajihassani, Abolfazl<sup>1</sup>, T. Schwarz<sup>3</sup>, and W. B. Rutter<sup>2</sup>. <sup>1</sup>Department of Plant Pathology, University of Georgia, Tifton, GA 31794. <sup>2</sup>Department of Entomology and Plant Pathology, North Carolina State University, Raleigh, NC 27695-7616. <sup>3</sup>USDA-ARS United States Vegetable Laboratory, Charleston, SC 294142.

Root-knot nematodes are insidious root parasites and are important constraints to the achievement of global food security. In recent years, nonchemical options have been sought to replace the loss of effective chemical products for control of these pests. The 'litchi' tomato (Solanum sisymbriifolium) has been used to manage potato cyst nematodes, and when used as a rootstock for cultivated tomatoes (Solanum lycopersicum) it is reported to provide resistance to biotic and abiotic stresses. A series of experiments were conducted under greenhouse conditions to examine the reaction of S. sisymbriifolium to infection by five major root-knot nematode species. Two cultivars White Star and Diamond and one germplasm line SIS Syn II of S. sisymbriifolium as well as susceptible tomato (Solanum lycopersicum cy. Rutgers) were grown and inoculated with 1,000 J2 of M. incognita race 3, M. arenaria race 1, M. haplanaria, and M. javanica. Plants were also infected with 10,000 eggs of *M. enterolobii* - a population each from North Carolina and South Carolina. Eight weeks after nematode inoculation, root systems were washed, weighed, and rated for galling severity and nematode reproduction using standard methods. The roots of all S. sisymbriifolium showed significant (P < 0.05) reduction in galling compared to the Rutgers control. The susceptible Rutgers control was an excellent host for all nematode species having a reproduction factor (RF, final population/initial population) ranging from 16.7 to 23.8. In contrast, significant variability was observed in the reproduction of different Meloidogyne species on different S. sisymbriifolium lines/cultivars. All 3 S. sisymbriifolium lines/cultivars were highly resistant to both M. haplanaria (RF = 0.02–0.24) and M. enterolobii (RF = 0.05–0.26). White Star (RF=0.13) and SIS Syn II (RF=0.08) were resistant to M. incognita, and SIS Syn II (RF=0.40) was resistant to M. arenaria. Interestingly, all 3 S. sisymbriifolium lines/cultivars were highly susceptible to M. javanica. The variable resistance patterns observed in S. sisymbriifolium to M. incognita, M. arenaria, M. haplanaria, and M. enterolobii indicate the presence of one or more potentially useful resistance genes. Further genetic studies are warranted to characterize the root-knot nematode resistance found in S. sisymbriifolium.

# CONTROL OF CITRUS NEMATODE, *TYLENCHULUS SEMIPENETRANS*, WITH OXAMYL, CADUSAFOS, AND BIOCONTROL AGENTS. **Hammam, Mostafa, M. M. M. Mohamed, and M. M. Abd-Elgawad**. Plant Pathology Department, Agricultural and Biological Research Division, Elbehooth St., National Research Centre, Dokki 12622, Giza, Egypt.

The effect of oxamyl, cadusafos, and selected biocontrol agents on *Tylenchulus semipenetrans* population densities and on lemon yield was investigated in field trial in Egypt. Eight treatments were applied under tree canopies as soil treatments in a randomized complete block design with ten trees as replicates per treatment in autumn. These were 1) indigenous *Heterorhabditis indica* at a rate of 25 infective juveniles/cm<sup>2</sup> on the soil surface, 2) *H. indica*-infected cadavers of *Galleria mellonella* last instar larvae applied at a rate of 10 insects/ tree beneath the soil surface, 3) and 4) oxamyl 24% L applied at 22.5 and 30 ml/ tree (= 3 and 4 litre/ Feddan) on the soil surface, 5) Cadusafos 10% Gr applied at 180 gm/ tree (= 23 Kg/ Feddan) on the soil surface, 6) Micronema\* (containing109 CFU/ml of *Serratia* sp., *Pseudomonas* 

sp., *Azotobacter* sp., *Bacillus circulans* and *Bacillus thuringiensis*) applied at 200 ml/ tree (= 27 litre/ Feddan) on the soil surface, 7) Apamax<sup>\*</sup> applied at 3 ml/ 7.5 litre of water per tree on the soil surface, and 8) untreated check. All agricultural practices were carried out as recommended. All treatments suppressed ( $P \le 0.01$ ) nematode male and second-stage juvenile population densities in soil as well as number of females on roots in both the following winter and spring. Accumulated lemon yield increased in all treatments compared to the control eight months after the treatments. The degree of increase in lemon yield was not consistent with the magnitude of nematode suppression in the different treatments. The highest yield was attained by Micronema followed by both Cadusafos and oxamyl (4 litre/Feddan) then by both oxamyl (3 litre/Feddan) and Apamax and finally by EPN applications. The experiment is continued for further estimation of lemon yield and nematode population levels to monitor the possible carryover effects of the treatments on future lemon yield.

MOLECULAR AND MORPHOLOGICAL CHARACTERIZATION OF *VITTATIDERA ZEAPHILA* FROM INDIANA. **Zafar Handoo<sup>1</sup>**, **A. M. Skantar<sup>1</sup> M. Kantor<sup>1</sup>**, **L. K. Carta<sup>1</sup>**, **J. Faghihi<sup>2</sup>**, **V. Ferris<sup>2</sup>**. <sup>1</sup>Mycology & Nematology Genetic Diversity & Biology Laboratory, USDA, ARS, BARC-West, Bldg. 010A, Rm. 111, Beltsville, MD 20705, USA. <sup>2</sup>Purdue University, Department of Entomology, 901 West State St. West Lafayette, IN 47907-2089.

In the summer of 2016, a field of corn (*Zea mays*) in Spencer County, Indiana was observed with heavily stunted plants. From the affected roots, a large number of cysts were recovered. Spencer county's geographic location is just across the Ohio River from Kentucky, approximately 200 miles away from Hickman County, Kentucky, where the nematode was previously reported. Soil samples were submitted to one of us (JF), who extracted the nematode cysts and sent them to the USDA-ARS, Mycology and Nematology Genetic Diversity and Biology Laboratory (MNGDBL), Beltsville, Maryland, for morphological and molecular identification. After fixation, the cysts and juveniles (J2) recovered from cysts were examined molecularly and morphologically. The cysts and second stage juveniles (J2) that were examined morphologically were consistent with the measurements of *Vittatidera zeaphila*, the goose cyst nematode originally described from Tennessee, USA, in 2010. The molecular analysis of J2 using ITS and 28S molecular markers showed a 99-100% similarity with sequences deposited deposited in GenBank as *V. zeaphila*. Molecular markers previously unreported with the original isolate were analyzed, including ribosomal small subunit (18S) rDNA, mitochondrial cytochrome oxidase I (COI), and nuclear heat shock protein 90 (Hsp90). Similarities to existing cyst nematode sequences are reported herein. To the best of our knowledge, this is the first report of *V. zeaphila* in Indiana.

ASSESSMENT OF COTTON AND SOYBEAN ROOT PROTECTION BY FLUOPYRAM-TREATED SEED AGAINST *MELOIDOGYNE INCOGNITA*. Hawk, Tracy<sup>1</sup> and T. R. Faske <sup>2</sup>. <sup>1</sup>Department of Plant Pathology, University of Arkansas, Fayetteville, AR 72703. <sup>2</sup>Department of Plant Pathology, Lonoke Extension Center, University of Arkansas, Lonoke, AR 72086.

The southern root-knot nematode, Meloidogyne incognita, is one of the most economically important plant- parasitic nematodes that affect cotton and soybean production in the southern United States. Nematicides continue to be an important part of an integrated management system in cotton and soybeans, however, with limited options available, new nematicides are needed. One example of a new nematicide is the compound fluopyram. While fluopyram has been shown to affect nematode motility, there is limited information on its effect to suppress nematode development in root systems. The objectives of this research were to determine the effect of seed-applied fluopyram on *M. incognita* life cycle and to determine the field efficacy of fluopyram-treated seed in a *M. incognita* infested field. Fluopyram-, abamectin-, or non-nematicide-treated soybean and cotton seeds were planted in M. incognita infested soil and maintained in a greenhouse. Plants were sampled at 7, 14, 21, 28, and 35 days after planting and nematodes were stained to facilitate counting using a stereoscope. Nematode root galls and reproduction were quantified at later sampling dates. In one cotton experiment, numerically fewer juveniles were observed inside roots from fluopyram-treated seedlings compared to the non-nematicide treated control. Nematode galling and reproduction were also reduced as result of the fluopyram-treated cotton seed. Similar observations were observed with abamectin-treated cotton seed. In the sovbean trial, there was a similar a trend at 7 and 21 days with significantly fewer penetrating nematodes in fluopyram-treated seedlings compared to the non-nematicide control; however, there was no effect of treatment at 14 days. There were significantly fewer galls and less reproduction 35 days after planting in fluopyram-treated seedlings compared to the control. In the field study, there were no statistical differences among treatments for either percent root system galled or yield, however, fluopyram- treated seeds had numerically greater grain yield and lower percent galled roots compared to both abamectin-treated and the non-nematicide control. For both greenhouse and field trials, the degree of seedling protection provided by fluopyram- treated seeds was similar to that of abamectin.

#### EVALUATING IMMUNE RESPONSES IN LEAF AND ROOT TISSUES WITH ROOT-KNOT NEMATODE EGG DERIVED ELICI-TOR(S). **He, Jiangman, D. Godinez-Vidal, D. Zhou, M. Teixeira, and I. Kaloshian**. Department of Nematology, University of California, Riverside, CA 92521.

Plants perceive invading pathogens and parasitic nematodes through plasma membrane-localized receptors that recognize conserved molecular patterns from these organisms to activate pattern-triggered immune (PTI). Among these responses, are oxidative burst and accumulation of reactive oxygen species (ROS), activation of mitogen-activated protein (MAP) kinases, upregulation of transcription factors and defense marker genes. Typically, the majority of these defense responses have been evaluated in leaf tissues. Some of these responses are transient and timing of the pathogen exposure/infection is important to document the proper responses. The nature of nematode infections does not lend itself to such time sensitive immune response assessments. To evaluate PTI responses against the root-knot nematode *Meloidogyne incognita*, we used nematode egg extracts as an elicitor for defense. To quantify these responses, we utilized wild-type *Arabidopsis thaliana* and allelic mutants that exhibit enhanced resistance to root-knot nematodes (*ern1-1 and ern1-2*). To obtain *M. incognita* egg extracts, eggs were bleach treated, floated on a 35% sucrose cushion and ground in liquid nitrogen. Exposing Arabidopsis leaf or root tissues to the egg extracts elicited PTI responses. Using Western blot analysis, enhanced phosphorylation of MAPK3 and MAPK6 was detected in the roots of the *ern* mutants compared to the roots of the wild-type Col-0 treated with the egg extracts. Similarly, higher levels of ROS burst were detected in leaf tissues, using chemiluminescence-based bioassay, and in roots, using DAB (3,3'-Diaminobenzidine)

staining, of the *ern* mutants compared to the wild type. Our results demonstrated that extracts of root-knot nematode eggs, and likely egg extracts of additional nematode species, could be used as an immune elicitor in both above ground and below ground plant tissues providing a user-friendly tool to assess host responses against nematodes.

WIDE DISTRIBUTION OF WHITE SOYBEAN CYST NEMATODE, *HETERODERA SOJAE*, IN KOREA. **Heonil, Kang<sup>1</sup>, H. Ko<sup>2</sup>, G. Han<sup>3</sup>, D. Kim<sup>4</sup> and I. Choi<sup>1,4</sup>.** <sup>1</sup>Pusan National University, Miryang 50463, Korea. <sup>2</sup>Crop Protection Division, National Institute of Agricultural Sciences, Wanju 55365, Korea. <sup>3</sup>Seoul National University, Seoul 00826, Korea. <sup>4</sup>Nematode Research Center, Pusan National University, Miryang 50463, Korea.

Soybean cyst nematode (*Heterodera glycines*, SCN) is one of the most harmful pathogen of soybean (*Glycine max* (L.) Merr.) worldwide. In 2016, a new soybean parasitic cyst nematode, *Heterodera sojae* was found from the roots of soybean plants in Korea. To investigate the distribution and population density of *H. sojae*, 943 soil samples were collected from soybean fields in 2018. Cysts were detected from 343 samples (36.4%). Among them, 227 samples (66.2%) were *H. glycines*, 95 samples (27.7%) were *H. sojae* and 21 samples (6.1%) were infested with both *H. sojae* and *H. glycines*. Population density of *H. sojae* was 71 cysts and 110 second-stage juveniles per 300 cm<sup>3</sup> soil, while *H. glycines* was 55 cysts and 100 second-stage juveniles per 300 cm<sup>3</sup> soil. It confirms that *H. sojae* is widely distributed in soybean fields in South Korea.

COMPARATIVE HATCHING BEHAVIOR OF *HETERODERA SOJAE* AND *H. GLYCINES* IN VITRO. **Heonil, Kang<sup>1</sup>, G. Kwon<sup>1</sup>, J.** Seo<sup>1</sup>, N. Park<sup>2</sup>, D. Kim<sup>2</sup>and I. Choi<sup>1,2</sup>.<sup>1</sup>Pusan National University, Miryang 50463, Korea. <sup>2</sup>Nematode Research Center, Pusan National University, Miryang 50463, Korea.

Relatively new soybean parasitic cyst nematodes, *Heterodera sojae*, white soybean cyst nematode, was found in 2016 and is widely distributed in soybean fields in Korea in a ratio of 3:7 (*H. sojae* : *H. glycines*). Observation indicated that cyst of *H. sojae* has thicker cuticle denser musilages around eggs than *H. glycines*, thus we speculated in differences in biology between two species. Cysts were treated with deionized water, 3.0mM ZnCl<sub>2</sub>solution and 1.3 RGH soybean root diffusate in 24 cell culture plates and incubated at 15°C, 20°C, 25°C and 30°C for 10 days. After 10 days of incubation, more juveniles hatched from *H. glycines* than *H. sojae*. Hatching ability of *H. sojae* was 0.1% at 15°C, 2.8% at 20°C, 8.2% at25°C and 6.2% at30°C in soybean root diffusate whereas 1.5% at15°C, 5.8% at 20°C, 10.7% at25°C and 4.6% at 30°C for *H. glycines*. In ZnCl<sub>2</sub> solution, 0.1% at 15°C, 0.7% at 20°C, 9.8% at 25°C and 9.2% at 30°C for *H. sojae*, while 5.4% at 15°C, 24.0% at 20°C, 34.6% at 25°C and 7.6% at 30°C in ZnCl<sub>2</sub>solution for *H. glycines*. Indeionized water, both species hatched little (0.01% at 15°C, 0.3% at 20°C, 0.5% at 25°C and 0.6% at 30°C for *H. sojae*, 0.2% at 15°C, 2.7% at 20°C, 3.8% at 25°C and 2.9% at 30°C for *H. glycines*). This results showed that two species, *H. sojae* and *H. glycines* has different hatching responses.

TECHNIQUES AND INSIGHTS INTO PLANT-PARASITIC NEMATODE-ASSOCIATED MICROBIOMES. Hesse, Cedar N.<sup>1</sup>, A.B. Peetz<sup>1</sup>, S.K. Wasala<sup>2</sup>, N. Carleson<sup>1</sup>, D.K. Howe<sup>2</sup>, D.R. Denver<sup>2</sup>, and I.A. Zasada<sup>1</sup>. <sup>1</sup>USDA ARS, 3420 NW Orchard Ave., Corvallis, OR 97330. <sup>2</sup>Department of Integrative Biology, Oregon State University, 3029 Cordley Hall, Corvallis, OR 97331.

Microbial interactions with multicellular organisms have been increasingly demonstrated as important factors in understanding many biological systems. Despite the advancement in genomic methods for investigating plant-nematode interactions, few studies have looked at the role microorganisms may play in the infection and life cycle of plant-parasitic nematodes. In an effort to better understand the naturally occurring bacterial communities associated with plant-parasitic nematodes, we utilized two high-throughput sequencing-based methods to investigate the microbiomes of a variety of nematode species. The first method, a 16S rDNA amplicon sequencing study of multiple life-stages of root knot nematodes (*Meloidogyne* spp.), showed infective juvenile (J2) microbiomes largely dominated by Proteobacteria, however 49 bacterial communities differed by geographic location from which nematodes were collected. *Meloidogyne* spp. egg and hatched J2 microbiomes were less diverse than soil-isolated J2, indicating the potential for vertical transmission of microbial associates. The second study involved the data mining of bacterial sequences and marker genes from low- coverage genome shotgun sequencing of a variety of plant-parasitic nematodes. "Blobplot" visualization of *de novo* genome assembly information allowed for detection of high-abundance bacterial genomic fragments that were co- extracted from nematodes.

## PHOTO-BIOLOGY: USING LOW-ENERGY UV LIGHT TO IDENTIFY NEMATODES. **Hiltpold, Ivan<sup>1</sup>, A J. Owens<sup>2</sup>, A. Ragone<sup>2</sup>**. <sup>1</sup>University of Delaware, Entomology and Wildlife Ecology, Newark, DE, USA. <sup>2</sup>Speckciton Bioscience LLC, Wilmington, DE, USA.

Identification of nematodes can be especially challenging and requires advanced skills. Preparing slides for morphological identification involves several steps and the use of harmful chemicals in addition to human dexterity, and good microscopy facilities. The recent development of several specific primers makes molecular identification and quantification very accurate. However, the extraction of DNA and its molecular amplification can still be problematic for some laboratories, both financially and technically. Here, we explored a new avenue in nematode identification. Based on the assumption that photo-biological signatures are species specific, we have exposed *Heterorhabditis bacteriophora*, *Steinernema feltia*, and *S. carpocapsae* to various light wavelength (visible, UV and deep-UV spectrums) and recorded their spectral signatures. Using a hand-held spectrometer, we measured an optimal fluorescence of the three species using a 375 nm UV laser. This technique allowed a quick and repeatable differentiation between the three tested species. In addition, the use of deep-UV (200-300 nm) permitted an accurate evaluation of the nematode density in the samples.

Predictive model allow the identification to the species level of the three tested nematodes. In addition, various mixture of the three tested species were tested to asses this approach with more complex samples. This technique still requires refinements but it provides a very quick, low-cost and low-labour technique to accurately identify and quantify entomopathogenic and/or other nematode species.

IDENTIFICATION OF SUITABLE *MELOIDOGYNE* SPP. HOUSEKEEPING GENES. Hu Weiming, P. M. DiGennaro. University of Florida, Department of Entomology and Nematology, Gainesville FL, 32611.

Gene expression studies often require reliable housekeeping (HK) genes to accurately capture expression levels under given conditions. This is especially true for root-knot nematode (RKN; *Meloidogyne* spp.), whose drastic developmental changes are strongly dependent upon their environment, including plant biology. Here we utilized a publicly available *M. hapla* RNASeq database to identify 39 putative HK genes throughout the nematode lifecycle, and then tested on *M. incognita* in order to develop a small library of putative HK genes suitable to most species of RKN. Seven of those candidate HK genes were selected for validation based on lack of secretion signal and ease of primer design. The expression of those seven genes were quantified by doing qPCR at the early infection stage and adult stage with diurnal treatment, and the whole life cycle of *M. incognita* which included egg and naive infective juveniles. Two algorithms, geNorm and Normfinder, identified four genes (*Poly, PTP, Skinase*, and Minc01079) that are stably expressed throughout the entire life cycle of RKN. Importantly, while we identified *Actin* as a putative HK gene from our RNASeq analysis, the qPCR results did not validate stable expression throughout the nematode life cycle. This study successfully validated four suitable HK genes using both RNASeq data and standard qPCR methods across two species of RKN and provides a list of HK genes likely suitable for species of RKN with available genomes. Future gene expression studies on RKN will greatly benefit from the HK genes suggested by this study.

PATHOGENICITY OF *MELOIDOGYNE INCOGNITA* AND TWO FUNGAL PATHOGENS: *FUSARIUM OXYSPORUM* AND *PYTHI-UM APHANIDERMATUM* ON SOYBEAN (*GLYCINE MAX L. MERR.*). **Charles Chidozie Iheukwumere<sup>1</sup>, O. J. Apeji<sup>2</sup> and C. I. Iheukwumere<sup>3</sup>**. <sup>1</sup> Department of Biological Sciences, University of Agriculture Makurdi, Benue state, Nigeria; <sup>2</sup> Formerly of Genetic Resources Unit (GRU) International Institute of Tropical Agriculture (IITA), Ibadan Nigeria; <sup>3</sup> Centre for Entrepreneurship Development, University of Agriculture Makurdi, Benue state. Nigeria.

Soybean (Glycine max L. Merr.) with its nutritional attributes and applications in family delicacies like soymilk, soyogi, moin-moin, puff puff, akara e.t.c., makes it a very healthy nutritional grain. Useful phytochemicals in soybeans provide immunity against cardio-oxidative reactions; act as anti-clotting agents and antioxidants while exerting anti- inflammatory effects in the body. Unfortunately, optimized local production is threatened by virus, nematodes and fungi attacks. The root-knot nematode Meloidogyne incognita, Chitwood, Fursarium oxysporum f.sp. vasinfectum, Pythium aphanidermatum in single and combined infections, constitute some of the foremost limiting influences on the growth and yield of the crop. This work investigated effects of single and combined infections of each pathogen on growth and yield components including Bradyrhizobium root nodules in screen house and field experiments. The most significant single reduction was by the nematode; while P. aphanidermatum exerted the most significant growth suppressions in comparison with that caused by F. oxysporum only. Significantly higher growth and yield suppressions occurred with P. aphanidermatum than with F. oxysporum in simultaneous or successive infections with the nematode and each of the fungi pathogens. Reductions by combination of the nematode and respective fungus, were not statistically different in the screen house experiment but were for most parameters in the field trial. In both experiments, number of galls and second stage juveniles of the nematode were more significantly reduced in combined than in single infection with only nematode. Reductions in the above parameters were significantly higher for the nematode and P. aphanidermatum complex than with the nematode and F. oxysporum. These outcomes established the threat status and dynamics of these pathogens to the optimization and sustainability of soybean cultivation and propagation in a country like Nigeria. This scenario further compounds the challenge of attaining food sufficiency in protein nutrition in Nigeria.

VETIVER SHOOTS AS SOIL AMENDMENTS FOR SUPPRESSION OF *MELOIDOGYNE INCOGNITA*. Jindapunnapat, Kansiree<sup>1,2,3</sup>, S. L. F. Meyer<sup>3</sup>, M. H. MacDonald<sup>3</sup>, N. D. Reetz<sup>3</sup>, D. J. Chitwood<sup>3</sup>, E. P. Masler<sup>3</sup>, N. Soonthornchareonnon<sup>4</sup>, M. J. Camp<sup>5</sup>, A. Sasnarukkit<sup>1</sup>, and B. Chinnasri<sup>1</sup>. <sup>1</sup>Department of Plant Pathology, Faculty of Agriculture, Kasetsart University, Bangkok, Thailand. <sup>2</sup>Center for Advanced Studies for Agriculture and Food, KU Institute for Advanced Studies, Kasetsart University, Bangkok, Thailand (CASAF, NRU-KU, Thailand). <sup>3</sup>USDA, ARS, Mycology and Nematology Genetic Diversity and Biology Laboratory, Northeast Area, Henry A. Wallace Beltsville Agricultural Research Center, Beltsville, MD 20705, USA. <sup>4</sup>Department of Phamacognosy, Faculty of Pharmacy, Mahidol University, Bangkok, Thailand. <sup>5</sup>USDA, ARS, Statistics Group, Office of the Director, Northeast Area, Henry A. Wallace Beltsville Agricultural Research Center, Beltsville, MD 20705.

Vetiver grass (*Vetiveria zizanioides*) is widely planted in tropical areas worldwide. The many uses of this grass include application of shoots as green manures or mulches to conserve water, improve soil and reduce weeds. Vetiver also produces compounds that are active against nematodes, and some cultivars are resistant to *Meloidogyne* spp. (root- knot nematodes). In greenhouse trials, the commercially available vetiver cv. Sierra was resistant to *Meloidogyne incognita*. To determine effects of vetiver shoot soil amendments on vegetable crops, greenhouse studies were conducted with cucumber, pepper and tomato seedlings transplanted into soil that had been amended with fresh, chopped vetiver shoots. Results depended on vegetable plant species, time from amendment to transplant, and amount of vetiver green manure. Cucumber seedling response varied from no significant effect to some phytotoxicity, while tomato seedlings had lower shoot heights and root fresh weights in higher vetiver amendment rates. Pepper roots were generally smallest when seedlings were transplanted into amended soil 3-4 weeks after vetiver amendment, as opposed to transplanting sooner after amendment application. Vetiver soil amendments were also tested in the greenhouse for suppression of *M. incognita* on cucumber. Amendment rates were 1%, 3%, 5% and 10% g fresh vetiver shoots/g dry soil (weight/weight). Only the 10% amendment rate consistently suppressed nematodes on cucumber roots; numbers of eggs/g root were reduced by 46% to 67%, compared with controls without vetiver amendment. Further studies would indicate whether amending soil with vetiver as part of a broader strategy for nematode suppression would contribute to root-knot nematode management.

COMPARISONS OF FLUOPYRAM AND FLUENSULFONE TO MANAGE *GLOBODERA TABACUM SOLANACEARUM* ON TOBAC-CO IN VIRGINIA. Johnson, C.S<sup>1,2</sup>, Noah Adamo<sup>1,2</sup>, and R.S. Irby<sup>1</sup>. <sup>1</sup>Virginia Tech Southern Piedmont Agricultural Research & Extension Center (AREC), Blackstone, VA 23824. <sup>2</sup>School of Plant and Environmental Sciences, Virginia Tech, Blacksburg, VA 24061.

Field experiments were conducted in 2009-2011 and 2014-2018 to compare potential new nematicides with standard products for control of Globodera tabacum solanacearum (Tobacco Cyst Nematode or TCN) on flue-cured tobacco. All trials were conducted in pre-selected, naturally-infested fields at Virginia Tech's Southern Piedmont AREC to maximize the size and uniformity of initial TCN populations. Each was arranged in a randomized complete block design with four to six replications. TCN population densities were estimated from soil samples collected before treatment application, mid-season, and after final crop harvest. Plant samples were also collected 35-74 days after transplanting to enumerate TCN juveniles in 1g subsamples of feeder roots. Cured leaf yield was estimated from leaves harvested sequentially as they ripened. Nematode control arising from transplant water application of fluopyram was compared in 2014-2018 to that in untreated control plots or after fumigation with 1,3-dichloropropene (1,3-D or Telone II). Application of fluopyram alone or combined with imidacloprid reduced TCN juvenile numbers compared to the untreated control in 2014-2016, but not in 2017 or 2018, when juvenile numbers in roots were generally much lower for all treatments. Total TCN juveniles/g root were lower after fluopyram treatment than in 1,3-D treated plots in 2014 and 2015, but juvenile numbers were similar in 2016-2018. Soil fumigation with 1,3-D increased yield compared to the untreated control in 2014-2016, but not in 2017 or 2018. Mean yields were numerically higher for fluopyram treatments in 2014-2017 compared to the untreated control, but these trends were only statistically significant in 2015 and 2016. Trials in 2009-2011 compared results from treatment with fluensulfone formulated as MCW-2 to those observed in untreated control plots or after application of aldicarb or 1,3-D; 2016-2018 tests evaluated the nematicidal activity of fluensulfone formulated as Nimitz. All fluensulfone applications were made before planting, either as a broadcast treatment or in 30-61 cm bands centered on the rows to be planted. Broadcast use of fluensulfone reduced TCN juveniles/g root only in 2010, and only at the highest rate - 8 kg a.i./ha. Treatment yields were highly variable and trends in yield among treatments were never statistically significant. Numbers of TCN juveniles/g root were numerically lower for both banded and broadcast-applied fluensulfone treatments in 2010 and 2018, but only the 8 kg a.i./ha fluensulfone rate and Telone treatments in 2010 were significantly different from the control. Flue-cured tobacco yields after banded application of fluensulfone were not significantly different from the untreated control in 2010, 2017 or 2018. These multi-year results suggest that neither fluopyram nor fluensulfone will offer TCN control comparable to 1,3-D, but that fluopyram may offer more benefits to growers than fluensulfone, at least under the experimental conditions and with the application methods used in these experiments.

FIRST REPORT OF *MELOIDOGYNE HISPANICA* ON ORIENTAL MELON (*CUCUMIS MELO* VAR. *MAKUWA*) IN KOREA. Jongmin, Seo<sup>1</sup>, H. Kang<sup>1</sup>, G. Kwon<sup>1</sup>, N. Park<sup>2</sup>, D. Kim<sup>2</sup> and I. Choi<sup>1,2</sup>. <sup>1</sup>Pusan National University, Miryang 50463, Korea. <sup>2</sup>Nematode Research Center, Pusan National University, Miryang 50463, Korea.

Root-knot nematodes are wide spread and cause much damage to oriental melon (*Cucumis melo* var. *makuwa*) in Korea. We found rootknot nematodes on oriental melon roots in plastic film house. This root-knot nematodes species is *M. hispanica* as the results estrase isozyme pattern analysis using phastsystem. This is the first report of *M. hispanica* from oriental melon. Polymerase Chain Reaction (PCR) amplification of the region between COII and 16S rRNA of the mitochondrial DNA produced a single fragment approximately 1.7 kb. Amplified PCR products were not digested with *HinfI* land *MnII* enzyme. Amplification with TRNAH and MRH106 produced fragments of 557bp; further digestion with *HinfI* generated 445bp and 112bp fragments and digestion with *MnII* produced three fragments at 346bp, 134bp and 77bp which confirmed the species is *M. hispanica*. Mitochondrial genome of *M. hispanica* is circular, with a sequenced size of 16,649 base pair. Compared with other plant-parasitic nematodes genera, this genome is only slightly smaller than that of *Pratylenchus vulnus* (21,656 bp). All genes (12PCGs, 2 rrn genes and 22 trn genes) were encoded on the same strand. The nucleotide composition for the mt genome was 83.6% AT and 16.4% GC for this species. Compared to other major *Meloidogyne* species (*M. incognita*, *M. arenaria* and *M. javanica*) this mt genome is smaller, due to the difference of non- coding regions (NCRs). *M. hispanica* had two large NCRs with sizes of 1,060 bp (NCR3) and ~ 3.1 kb (NCR1). NCR3 was present in the tropical mitotic parthenogenetic species. The largest NCR (NCR1) in *M. hispanica* was shorter than other major *Meloidogyne* species (~5.1-5.6kb in *M. incognita*, ~5.7kb in *M. arenaria*, ~6kb in *M, janavica*). The NCR of the mt genome in animals called the control region, which may act as the origin of replication and as a promoter for transcription initiation in animal mitochondrial DNA.

COMPOSTED CHICKEN MANURE-BASED SOIL HEALTH MANAGEMENT IN SELECTED VEGETABLE VALUE CHAINS ON SMALLHOLDER FARMERS IN MALAWI. **Kakaire, Stephen<sup>1</sup>, K. Njira<sup>2</sup>, L. Chilasa<sup>2</sup>, D. Chipeta<sup>3</sup> and H. Melakeberhan<sup>1</sup>.** <sup>1</sup>Michigan State University (MSU), Dept. of Horticulture, 1066 Bogue Street, East Lansing, MI 48824 USA. <sup>2</sup>Lilongwe University of Agriculture and Natural Resources (LUANAR), Dept. of Crop and Soil Sciences, P. O. Box 219 Lilongwe, Malawi. <sup>3</sup>Land O'Lakes (LOL) Inc., Private Bag A148, Lilongwe, Malawi.

The adverse effects of the biophysicochemical degradations of Malawi soils on food insecurity and ecosystem sustainability are well established. The goal of this LOL, MSU and LUANAR collaborative research is to develop technologies that alleviate the soil health management challenges. To achieve the goal, farm demonstrations were conducted using chili (*Capsicum annuum*), onion (*Allium cepa*), potato (*Solanum tuberosum*), and tomato (*Solanum lycopersicum*) as models in Mchinji, Lilongwe, Dedza and Mangochi districts. At each location, the four vegetables received either zero (check) or varying rates of a combination of composted chicken manure and recommended inorganic fertilizer (Compound D) at planting and Calcium Ammonium Nitrate side dress at three to five weeks after transplanting chilies and tomato or postemergence of potato and onions. These amendments delivered either 0 Kg of N (check), the recommended rate 92Kg (1X N), or 138Kg (1.5X N). Each treatment was replicated four times, giving a total of 192 plots across locations. Nematodes were analyzed from 100 cc sub-samples at-planting, mid-season and at harvest and soil physiochemistry at harvest. Nematodes were enumerated at MSU. The results varied by crop and location, and the controls performed poorly compared to the 1X and 1.5X N rates. The soil food web data indicated generally Quadrant C across crops and locations, an improvement from Quadrant D before the experiment was initiated. The 1X and 1.5X N rates yielded better than the control. Percent organic matter (%OM) and N were very low and varied by location and crop, with no significant amendment effect. Total nematode densities varied by crop and location. The optimum amendment rate is unknown and more time may be required for this to be established.

INTEGRATED NEMATODE AND SOIL HEALTH MANAGEMENT IN THE WESTERN HIGHLANDS OF GUATEMALAN POTA-TO PRODUCTION SOILS: I - SIMILARITIES AND DIFFERENCES IN SOIL FOOD WEB STRUCTURE AND FUNCTION. Kakaire, Stephen<sup>1</sup>, A. Sanchez-Perez<sup>2</sup>, B. S. Sipes<sup>3</sup>, C-L. Lee<sup>4</sup>, A. Sacbaja<sup>2</sup>, C. Chan<sup>3</sup>, and H. Melakeberhan<sup>1</sup>. <sup>1</sup>Department of Horticulture, and <sup>4</sup>CANR Statistical Consulting, Michigan State University, East Lansing, MI 48824 USA. <sup>2</sup>Faculty of Agronomy, University of San Carlos, Guatemala City, Guatemala, <sup>3</sup>University of Hawaii at Manoa, Honolulu, HI 96822 USA.

As part of the USAID's Horticulture Innovation Laboratory goal to alleviate nutrition and food insecurity, this collaborative research was conducted in smallholder potato production areas in Huehuetenango and Xela in the Highlands of Guatemala. These regions have soil health and plant-parasitic nematode management problems. The Huehuetenango region is at 3,200 m to 3,353 m altitude laying over Mollisol soil group (class) and the Xela region is at 2,896 m over Andisols. An experiment was conducted in each region over two years to answer multiple questions. In each region, the effects of amending soils either with or without bio-mix (BioCopia) and 0, 318, or 454 kg of composted chicken manure at eight locations were tested. The bio-mix consisted of Guatemalan isolates of Purpureum and Bacillus applied at 1.8 kg/m<sup>2</sup> to suppress harmful nematodes. This presentation reports on the effects of these treatments on nematode community structure (NCS), potato yield and percent organic carbon (C) and nitrogen (N), the combination of which indicates the overall soil health conditions. Nematodes were extracted from 100 cm<sup>3</sup> of soil, fixed in double TAF solution at USAC and enumerated. Nematodes were identified into trophic and colonizer-persister (c-p) groups and data computed to reveal the soil food web (SFW) structure and function. Soil C and N were measured. There was no significant treatment effect on either the enrichment or structure scales of the SFW during the growing season at either location. The only exception was that the SFW structure was different between years in the high rate without the bio-mix. The overall SFW structure of both soil groups indicates Quadrant C- needing biological activity for nutrients to be released. Herbivores and bacterivores were the abundant trophic groups and c-p 1s were the least abundant in both soil groups, suggesting that the soils have similar SFW structure. Potato yield was slightly higher in the Andisol fields; whereas, C:N ratio was higher in the Mollisol fields. The data suggest that the soils may have different SFW functions, the cause-and-effect of which is yet to be determined.

FIRST REPORT OF *PUNCTODERA STONEI* FROM UNITED STATES. **Mihail Kantor<sup>1</sup>Z. A. Handoo<sup>1</sup>, A. M. Skantar<sup>1</sup>, N. M. Wade<sup>2</sup> and R. E. Ingham<sup>2</sup>**. <sup>1</sup>Mycology & Nematology Genetic Diversity & Biology Laboratory, USDA, ARS, BARC-West, Bldg. 010A, Rm. 111, Beltsville, MD 20705, USA. <sup>2</sup> Botany and Plant Pathology Nematology Research Program & Extension Nematode Testing Service, Oregon State University, Corvallis, Oregon.

In the spring of 2019, a soil sample collected from a golf course from Southern Oregon (Brandon, OR), was received by the USDA-ARS, Mycology and Nematology Genetic Diversity and Biology Laboratory (MNGDBL), Beltsville, Maryland for species identification. A large number of cysts and juveniles (J2) were separated from soil by sieving and Baermann funnel extraction and were examined molecularly and morphologically. Morphologically, the cysts and second stage juveniles (J2) morphometrics were consistent with the measurements of *Punctodera stonei* originally described in Poland by Brzeski (1998). The molecular analysis of J2 using ITS and 28S molecular markers showed a 99 percent similarity with sequences deposited in GenBank as *P. stonei*. Three additional molecular markers not previously reported in a more recent study by Dobosz (2013) were analyzed. These markers included ribosomal small subunit (18S) rDNA, mitochondrial cytochrome oxidase I (COI), and nuclear heat shock protein 90 (Hsp90). Similarities to existing cyst nematode sequences are reported herein. To the best of our knowledge, this is the first report of *Punctodera stonei* in the United States.

MICROGRAVITY EFFECT ON ENTOMOPATHOGENIC NEMATODES' ABILITY TO FIND AND KILL INSECTS. **Kaplan, Fatma<sup>1</sup>**, **D. Shapiro-Ilan<sup>2</sup> and E. Sampson<sup>3</sup>**. <sup>1</sup>Pheronym, Inc., Davis, CA 95618. <sup>2</sup>USDA-ARS, Southeast Area Fruit and Tree Nut Research, Byron, GA 31008, <sup>3</sup>University of Florida, Gainesville, FL 32610.

Entomopathogenic nematodes (EPNs) are microscopic roundworms which are part of a healthy soil microbiota and pollinator-friendly biocontrol system for agricultural insect pest control. The nematodes kill insects with the aid of mutualistic bacteria. The nematodes enter insects through natural body openings or the insect cuticle, release their symbiotic bacteria, then the symbiotic bacteria infect and kill the insects. Once the insect host is consumed, a pheromone signal tells the nematodes that they are too crowded, need to leave, disperse and find a new insect to infect. Dispersal is a key feature contributing to EPNs' efficacy in finding and killing insects. Dispersal is strongly regulated by environmental stress such as vibration, sub optimal temperatures, barometric pressure changes, electrical fields, and pheromones. We hypothesize that microgravity is an environmental stress factor that changes pheromone production and affects EPN dispersal and efficacy. The microgravity experiment will be conducted at the International Space Station (ISS) National Laboratory (NL) and use EPN, *Steinernema feltiae*. We will investigate the followings; 1- to determine whether EPNs produce the same pheromone composition in microgravity vs. on Earth, 2- to test whether EPNs grown in microgravity have the same symbiotic bacteria as their parents on Earth, 3- the ability to forage infect reproduce and emerge, and 4- to determine whether EPNs can infect insect hosts in microgravity, and 5- how microgravity affects insect host physiology. We will present our experimental plans at the ISS NL.

ROLE OF MICRONUTIRENTS ON SYSTEMIC ACQUIRED RESISTANCE IN WATERMELON AGAINST *FUSARIUM OXYSPORUM* F. SP. NIVEUM AND *MELOIDOGYNE INCOGNITA*. **Karki, Kasmita<sup>1</sup>, A. Hajihassani<sup>1</sup>, T. Coolong<sup>2</sup>, S. Kousik<sup>3</sup>, and B. Dutta<sup>1</sup>. <sup>1</sup>Department of Plant Pathology, University of Georgia, Tifton, GA 31794. <sup>2</sup>Department of Horticulture, University of Georgia, Athens, GA 30602. <sup>3</sup>USDA-ARS, Charleston, SC 29414.** 

*Fusarium oxysporum* f. sp. *niveum* (FON) and southern root-knot nematode (RKN, *Meloidogyne incognita*) are devastating soilborne pathogens of cultivated watermelon in the southeastern United States. It has been reported that the concentrations of macro- and micronutrients in both the soil environment and plant tissues are associated with plant defense mechanisms. In this study, the role of controlled feeding of micronutrients [iron (Fe), manganese (Mn) and zinc (Zn)] via hydroponics on genes related to systemic acquired resistance [salicylic acid (SA) pathway genes (PR1, PR5, NPR1) and jasmonic acid (JA) pathway genes (VSP, PDF, and LOX)] in watermelon

seedlings was investigated. Micronutrients were fed to the plants at higher (3X) and lower (0.5X) levels of the recommended dose (X; Fe=3 ppm, Mn=1 ppm, and Zn=0.4 ppm) for 7 days and the expression levels of the above-mentioned systemic acquired resistance genes were evaluated. A sub-set of micronutrient-fed plants were inoculated with either FON or RKN or both on the 8<sup>th</sup> day of micronutrient feeding, and they were re-evaluated at 3-day post inoculation for the above-mentioned genes. Two independent trials were conducted in a completely randomized design with three replications per treatment. Results showed that JA-pathway genes (VSP and LOX) were upregulated in the plants with 7 days of micronutrient feeding. However, the expression of PR1, PR5, NPR1, and PDF genes were downregulated. Significant differences ( $P \le 0.05$ ) in PR1, PR5, PDF, and VSP expression were observed among micronutrient treatments. Upon pathogen (FON or RKN or both) inoculation, expression levels of JA and SA genes varied considerably ( $P \le 0.05$ ) for different micronutrient treatments. These observations indicate that micronutrient feeding in watermelon for 7 days can induce systemic acquired resistance and the expression of JA or SA pathway genes can potentially be affected upon infection by either FON or RKN or both, irrespective of nutrientfeeding.

EMPLOYMENT OF THERMOTHERAPY FOR NON-CHEMICAL MANAGEMENT OF NEMATODES OF STRAWBERRY TRANS-PLANTS. **Khanal, Churamani, H. Regmi, and J. Desaeger.** 14625 CR 672, Entomology and Nematology Department, University of Florida Gulf Coast Research and Education Center, Wimauma, FL 33598.

Each year more than 100 million strawberry transplants are shipped to Florida predominantly from Canada, northern California and North Carolina to be planted in the winter strawberry production fields. Pests and diseases, including many nematodes, are a major constraint for strawberry growers in Florida, and effective disease and nematode control has to start with clean planting material. However, strawberry transplants coming in to Florida often harbor pests, diseases and nematodes. Foliar nematode (Aphelenchoides besseyi), northern root-knot nematode (Meloidogyne hapla) and northern root lesion nematode (Pratylenchus penetrans) have all been found on strawberry transplants from out-of- state nurseries. Heat treatment or thermotherapy using aerated steam has been developed and proven effective against various pathogens in strawberries, and we are currently investigating its potential to control foliar, root-knot and lesion nematodes on strawberry transplants. Studies initially focused on evaluating the intrinsic temperature sensitivity of each nematode. This was done by adding 20 of each nematode species to 5 ml water in 20 ml glass vials that were placed in a water bath for 1, 5, 10, 30, 60, 120 and 240 min at 40, 44, 48 and 52°C. Exposure for 60 min or higher at 40°C paralyzed all three nematode species when examined immediately after heat treatment. Similarly, minimum exposure times to paralyze all nematode species at 44, 48 and 52°C were 60, 10 and 5 min, respectively. Nematodes were re-evaluated after 24 h, and some nematodes of each species that appeared paralyzed immediately after heat treatment had recovered from the heat stress. Overall, root-knot nematode was more sensitive to heat treatment followed by foliar and lesion nematodes. Heat treatment at 40°C for any of the exposure times was not enough to kill or paralyze 100% of all the nematode species after 24 h. The minimum exposure time and temperature combinations required to achieve 100% mortality of all three nematode species were 120 min at 44°C, 10 min at 48°C, and 5 min at 52°C. Greenhouse studies are currently under progress to determine efficacy of heated steam to kill the three nematode species in strawberry transplants. Because clean planting stock is the foundation of integrated pest management, thermotherapy can be used as a non-chemical method to kill the exotic nematodes of strawberry transplants before planting in the field.

FLUORESCENCE MICROSCOPY FOR EVALUATING PROGRESSION OF FUNGAL ANTAGONISM OF SOYBEAN CYST NEMA-TODE (*HETERODERA GLYCINES*) EGGS. **Kim, Dong-gyu**<sup>1</sup>, **D. Haarith**<sup>2</sup>, **S. Chen**<sup>2</sup>, **K. Bushley**<sup>3</sup>. <sup>1</sup>Biochemistry, Molecular Biology, and Biophysics, University of Minnesota, Minneapolis 55455. <sup>2</sup>Plant Pathology, University of Minnesota, Saint Paul 55108. <sup>3</sup>Plant and Microbial Biology, University of Minnesota, Saint Paul 55108.

Diverse soil fungi can antagonize the soybean cyst nematode (SCN; Heterodera glycines) at various stages of its life cycle through either direct parasitism and/or the secretion of a variety of nematostatic and/or nematotoxic secondary metabolites. Fungal biocontrol candidates offer an alternative to current environmentally damaging or economically unfavorable SCN management practices. Characterizing the dynamics and mechanisms of fungal antagonism towards SCN eggs may help commercialize fungi as biocontrol agents. Here we developed an affordable, rapid and insightful method of evaluating progression of fungal antagonism of SCN eggs through microscopy techniques. Monoxenic Race 3 SCN cysts were harvested from inoculated soybeans grown in cleanroom conditions with HEPA filters and treated with antibiotics to procure clean SCN cysts. Bioassays were conducted by inoculating clean Race 3 SCN cysts on colony peripheries of well-studied biocontrol candidate Pochonia chlamydosporia isolate 123, and an Ilyonectria sp. field isolate demonstrating in vitro SCN egg antagonism. Antagonized SCN eggs were released from the cysts and labeled with non- specific cellulose and chitin stain Calcofluor White M2R and cell death stain propidium iodide. Microscopy was conducted using a Nikon\* Eclipse 90i light microscope, using the appropriate excitations and filters to visualize fluorescence. We established Calcofluor White M2R stain as an effective means of visualizing fungal structures within the SCN egg, as well as potential points of interface with the nematode prey. Propidium iodide stain and differential interference contrast microscopy highlighted the nematotoxic effects of fungal antagonism manifesting as widespread cell death and lipogenesis respectively. Our results demonstrate distinct modes of SCN egg antagonism by fungal isolates and identify structural features of successful fungal antagonism through microscopy techniques. We anticipate our assay to become a tool for further research into interaction dynamics between fungi and plant-pathogenic nematodes. Furthermore, this technique may enable fungal isolates with complementary modes of antagonism against SCN to be combined in biocontrol trials.

DISCRIMINATION BETWEEN *HETERODERA SOJAE* AND *H. GLYCINES* USING COI AND ITS DNA BARCODING. **Hyoung-Rai** Ko<sup>1</sup>, Heonil Kang<sup>2</sup>, Insoo Choi<sup>2</sup>, Donggeun Kim<sup>2</sup> and Jae-Kook Lee<sup>1</sup>. <sup>1</sup>Crop Protection Division, National Institute of Agricultural Sciences, RDA, Wanju, 55365, Korea. <sup>2</sup>Department of Plant Bioscience, College of Natural Resources and Life Science, Pusan National University, Korea.

Soybean is one of important crop worldwide and the soybean cyst nematode is a major yield limiting factor. New soybean cyst nematode *H. sojae* was discovered in Korea in 2016 and is distributed widely in soybean fields together with *H. glycines* (ratio=3:7, unpublished data). The objective of this study was to discriminate between *H. sojae* and *H. glycines* using DNA barcoding technique. The sixteen cyst nematodes populations were obtained from soybean fields in Korea, and were identified as *H. glycines* (12 populations) and *H. sojae* (4 populations) by their cyst shape and followed analysis of COI and ITS DNA barcoding. In the COI and ITS analysis, *H. glycines* was clustered together with 'Schachtii' group including *H. schachtii, H. diverti, H. trifolii, H. betae, H. ciceri* while *H. sojae* was separately clustered with 'Cyperi' group including *H. cyperi, H. mothi, H. guangdongensis*, and *H. elachista*. This results were able to discriminate between *H. sojae* and *H. glycines* by COI DNA barcoding as well as ITS DNA barcoding.

THE POTATO CYST NEMATODE EFFECTOR RHA1B IS A UBIQUITIN LIGASE AND USES TWO DISTINCT MECHANISMS TO SUPPRESS PLANT IMMUNE SIGNALING. Kud, Joanna<sup>1</sup>, W. Wang<sup>2</sup>, R. Gross, Y. Fan<sup>2</sup>, L. Huang<sup>2</sup>, Y. Yuan<sup>2</sup>, A. Gray<sup>1</sup>, L.M. Dandurand<sup>1</sup>, and F. Xiao<sup>2</sup>. <sup>1</sup>Department of Plant, Soil and Entomological Science, University of Idaho, Moscow, ID, 83843, USA. <sup>2</sup>Department of Plant Sciences, University of Idaho, Moscow, ID, 83843, USA.

*Globodera pallida*, a plant-parasitic cyst nematode, causes vast losses in economically important crops such as potato and tomato. This sedentary endoparasitic nematode penetrates plant root system to gain access to nutrients inside the inner cortex through establishing a permanent feeding site, the syncytium. The development of this long-term biotrophic relationship with a host heavily relies on effector proteins injected into host cells through stylet to suppress plant innate immunity. Here, we report a novel *G. pallida* effector RHA1B highly upregulated in the early parasitic stage of infection. We demonstrate, using *in vivo* and *in vitro* ubiquitination assays, that RHA1B is an active E3 ubiquitin ligase that employs multiple host plant E2 ubiquitin conjugation enzymes to catalyze ubiquitination. We found that RHA1B promotes E3-dependent degradation of a broad range of NB-LRR immune receptors associated with effector-triggered immunity (ETI), including a potato Gpa2 protein conferring resistance to some isolates of *G. pallida*. Furthermore, RHA1B also blocks the flg22-triggered expression of *Acre31* and *WRKY22*, marker genes of pathogen-associated molecular pattern (PAMP)-triggered immunity (PTI), which does not require the E3 activity of RHA1B. Moreover, the virulent nature of RHA1B was confirmed by the enhanced susceptibility of transgenic potato plants ectopically overexpressing RHA1B. Overall, RHA1B is the first effector with ubiquitin ligase activity identified from eukaryotic pathogens infecting plants or animals. The ability of RHA1B to exploit multiple host E2s, and therefore, interfere with plant immunity in both E3-dependent and -independent manners, arms *G. pallida* with a unique advantage in parasitism.

SECONDARY METABOLITES OF NEMATODE SYMBIONT *PHOTORHABDUS L. SONORENSIS* (ENTEROBACTERIACEAE) SHOW A WIDE RANGE OFACTIVITY AGAINST THE ROOT KNOT NEMATODE, *MELOIDOGYNE INCOGNITA* (TYLENCHIDAE). **Kusakabe, Ayako<sup>1</sup>**, **Molnár, I.<sup>2</sup>**, **Stock, S. P.**<sup>1,3</sup>. <sup>1</sup>Department of Entomology, University of Arizona, 410 Forbes Building, Tucson, AZ 85721, United States. <sup>2</sup>Natural Products Center, University of Arizona, 250 E. Valencia Road, Tucson, AZ 85706, United States. <sup>3</sup>School of Animal and Comparative Biomedical Sciences, University of Arizona, 1140 E. South Campus Dr., Tucson, AZ 85721-0036, United States.

Insect pathogenic *Photorhabdus* bacteria, the natural symbionts of *Heterorhabditis* entomopathogenic nematodes, are considered a goldmine for the discovery and application of biologically active secondary metabolites (SMs) with antibacterial, antifungal, insecticidal, and nematicidal activities. In this study, we evaluated three metabolites that were isolated and purified from culture filtrates of *P. l. sonorensis* (strain Caborca). The chemical identification of active SMs was done by bioassay-guided fractionation. Spectral analyses identified two of these compounds as phenylpropanoids (AK1 and AK2) and one alkaloid (AK3). *In vitro* assays were carried out to assess the nematicidal activity of these SMs on the infective stage (second-juvenile stage or J2) of the root-knot nematode, *Meloidogyne incognita*. The activity of these SMs was also tested on four non-target nematode species: *Caenorhabditis elegans* (free-living bacterivore) and three entomopathogenic species, *Steinernema carpocapsae, H. bacteriophora, and H. sonorensis*. These compounds revealed different inhibitory activity ranging from a transient paralysis to death. AK1 and AK2 exhibited nematicidal activity to *M. incognita*. The LC<sub>50</sub> for AK1 was 64 µg/ml and 45 µg/ml for AK2. AK3 showed nematicidal activity to *M. incognita* and *C. elegans* at the two highest concentrations tested (300 and 400 µg/ml). At 60 to 200 µg/ml, AK3 induced reversible paralysis in both nematodes species. All entomopathogenic species tested were resistant to AK3. This work sheds light on ascertaining the potency of the *Photorhabdus*-derived SMs as nematicides.

COMPARATIVE TRANSCRIPTOMIC ANALYSIS OF *HETERODERA GLYCINES* FOR THE DISCOVERY OF VIRULENCE GENES. **Kwon, Khee – Man<sup>1</sup>, V. A. Lakshman<sup>1</sup>, W. G. Spollen<sup>2</sup>, M. N. Gardner<sup>3</sup>, and M. G. Mitchum<sup>1</sup>**. <sup>1</sup>Division of Plant Sciences and Bond Life Sciences Center, University of Missouri, Columbia, MO 65211. <sup>2</sup>Informatics Research Core Facility, University of Missouri, Columbia, MO 65211. <sup>3</sup>United States Department of Agriculture – Animal and Plant Health Inspection Service, Raleigh, NC 27606.

The soybean cyst nematode (SCN), *Heterodera glycines*, is a major pest of soybean primarily managed through deployment of resistant cultivars. However, the repeated use of the same resistant source has selected for virulent SCN populations that can reproduce and cause yield losses on resistant varieties. Despite this widespread problem with SCN virulence, it still remains unknown which nematode genes are involved in virulence. We have previously developed two unique SCN inbred populations that are highly adapted on two soybean lines that differ at the *Rhg4* locus, which harbors a serine hydroxymethyltransferase gene involved in SCN resistance. In this project, RNA-seq data generated from early parasitic life stages of these inbred populations were used to conduct a reference-based transcriptomic analysis with the aid of the SCN genome. The goal of this approach was to find differentially expressed (DE) genes, some of which may be potential virulence genes involved in overcoming the *Rhg4*-mediated resistance. Although both populations showed similar gene expression levels, there were 283 genes that were exclusively up-regulated, and 102 genes that were exclusively down- regulated in the population adapted to the resistant line. Moreover, 367 genes were up-regulated in the population adapted to the resistant line, which were also down-regulated in the population adapted to the susceptible line lacking the *Rhg4* resistance allele. These DE genes all have the potential to be involved in breaking the *Rhg4*-mediated resistance, and focusing on these genes will help prioritize candidate virulence genes for further molecular functional studies. Newly discovered nematode virulence genes may serve not only as molecular markers for rapidly diagnosing the virulence profile of SCN field populations, but also as novel targets for engineering nematode resistance to help develop more durable resistant soybean.

SOIL TYPE-DRIVEN FOODWEB DYNAMICS ASSOCIATED WITH *MELOIDOGYNE HAPLA* IN MICHIGAN VEGETABLE FIELDS. Lartey, Isaac<sup>1</sup>, T.L. Marsh<sup>2</sup> and H. Melakeberhan<sup>1</sup>. <sup>1</sup>Department of Horticulture. <sup>2</sup>Department of Microbiology and Molecular Genetics, Michigan State University, East Lansing, MI 48824, USA.

The northern root-knot nematode (NKRN) is a problem in the northern hemisphere vegetable cropping systems. Currently there are no commercially available resistant cultivars. The NRKN has parasitic variability that seems to be associated with soil types, and it occurs in varying soil health conditions. The agrobiological basis of NRKN parasitic variability remains unknown. The goal of this project is to understand how NRKN parasitic variability relates to the biological and physiochemical conditions in the environment in which it survives. The objective of this study is to establish any correlation between presence or absence of NRKN and soil health conditions, as indicated by nematode community, in different vegetable production regions of Michigan. To test the objective, 15 vegetable fields in three regions within the lower peninsula of Michigan (east, south-west and north-west) were selected. These fields represented muck and mineral soil types. In each of the fields, five  $25 \text{ m}^2$  area were flagged. Within each  $25 \text{ m}^2$ , one geo-reference flag was randomly marked on the rows to collect rhizosphere soil and another flag about 30 cm away in between rows to represent bulk soil. As a control, five sampling points were flagged in an adjacent non-agricultural field. Each sample consisted of approximately 1 liter of composite of 10 cores around a flag. Nematodes were extracted from 100 cc sub- sample of each sample and identified to trophic and colonizer-persister groups to determine soil food web structure and function. The presence or absence of NRKN was tested by planting two weeks-old tomato seedlings cv Rutgers into 300 cc sub-sample from each of the bulk soil samples. The assumption was that the soil from  $1 \text{ m}^2$  will have the same NRKN population. The experiment was set up in a greenhouse with an 8 h dark and 16 h day diurnal cycle. Twelve weeks later, seedlings were assessed for presence or absence of NRKN by gall index. Based on the Ferris et al soil food web model, the rhizosphere and bulk soil in the muck soils were primarily disturbed and the mineral soils primarily degraded. Those of the non-agricultural soils of both soil types were disturbed. In three of the nine mineral soil fields and in all the muck soils NRKN was present. How the presence of NRKN in degraded and disturbed soil condition relates to parasitic variability is being investigated.

## *IN VITRO* EVALUATION OF SALIBRO ON *MELOIDOGYNE INCOGNITA*. Lawaju, Bisho Ram and K.S. Lawrence. Department of Entomology and Plant Pathology, Auburn University, Auburn, AL 36849.

Plant-parasitic nematodes are responsible for significant crop damage and yield loss of agricultural production annually. Currently, studies are exploring the development of new biological or chemical compounds that target a specific plant-parasitic nematodes. Salibro (active ingredient: fluazaindolizine) is a new sulfonamide nematicide examined to determine its effects on Meloidogyne incognita egg hatch and juveniles (J2s) motility in vitro. Four different concentrations of Salibro (1, 5, 50, and 250 ppm of active ingredient) in aqueous solutions were evaluated and compared to sterile distilled water as a control. A randomized complete block design (RCBD) test was conducted in 12-wells tissue culture plates with 4 replications and the entire test was repeated for twice. Data was analyzed with SAS 9.4 using PROC GLIMMIX and LS-means were compared using Tukey-Kramer's method ( $P \le 0.05$ ). The result of *in-vitro* egg hatch assay indicated that high concentration (250 ppm) has slight inhibitory effect, while other tested concentrations of Salibro do not have any significant impact on egg hatch over 168 hours of exposure. In contrast, the lower concentration (1 ppm) was found to stimulate the hatching by 40% over the water control in same period of exposure. The effects of Salibro on M. incognita J2s motility was determined by visible symptoms of toxicological effects on the J2s in different time intervals after exposure. Healthy and normal J2s have rapid and sinusoidal movements whereas the affected exhibit twitching, coiling, and sluggish movements with characteristic J or Z shaped body postures. Dead J2s exhibited no movement and were straight rods. The M. incognita J2s motility was affected by Salibro at all rates with the affected J2s percentages ranging from 47% to 98% within 24 to 72 hours of exposure. At 120 hours of exposure, the M. incognita J2s motility was reduced to 73-91% with death occurring at 17-21% of the J2s at the test rates of 50 to 250 ppm of Salibro respectively. Nearly 100% of the M. incognita J2s were toxicologically affected or dead after 168 hours of exposure to the Salibro (50 to 250 ppm) while only 18% of the water control were affected or dead in the same period of time. The adverse effects of Salibro on M. incognita J2s were not reversible. The affected J2s did not recover after rinsing off the chemical and incubation in fresh water for 24 hours. The overall results indicated that the lower rate of Salibro at 50 ppm was adequate to reduce *M. incognita* J2s motility and the effect on the J2s was not reversible.

## INTERACTIONS IN THE PHYTOBIOME: UNDERSTANDING THE SYSTEM TO MANAGE PLANT HEALTH. Jan E. Leach, Department of Bioagricultural Sciences and Pest Management, Colorado State University, Fort Collins, CO 80523-1177.

Phenotypic responses of plants to biotic and abiotic stresses are frequently studied as the outcome of interactions between plants and one or two species of microbes or a single abiotic stress. However, in the phytobiome, plant health and productivity is impacted by simultaneous interactions among multiple organisms and the environment, and frequently, the responses to these interactions are distinct and would not be predicted from studying less complex systems. In this presentation, examples of multitrophic interactions among microbes, insects, plants and the environment will be used to demonstrate the difficulty in predicting outcomes from simultaneous stresses. Developing successful and sustainable crop improvement and management strategies for the future will benefit from studying these interactions as a system.

# COMPARISON OF NEMATICIDAL EFFICACY BETWEEN DIFFERENT SPECIES OF THE SAME GENUS WITH SAME HOST PLANT CYST NEMATODES. **D.W. Lee<sup>1</sup>**, **M.G. Jeong<sup>1,2</sup>**, **Md. F. Kabir<sup>1</sup>**. <sup>1</sup>Department of Ecological Science, Kyungpook Nnational University, Sangju, Gyeongbuk, Republic of Korea 37224. <sup>2</sup>Research Institute, NongHyup Chemical, Okcheon, Chungbuk, Republic of Korea 29008.

In Korea, *Heterodera glycines* and *H. sojae* are the same genus of cyst nematode that damage soybean, and *H. schachtii* and *H. trifolii* are the same genus of cyst nematode that damage Chinese cabbage. This study was carried out to compare the effects of nematicides on nematodes of the same genus damaging the same plants. The nematicidal activity of two nematodes (*H. glycines* and *H. sojae*) larvae

affecting soybean showed no difference for the four test nematicidal compounds (Fluopyram SC, Fosthiazate SL, Hydrogen peroxide, and Imicyafos SL) in laboratory. However, in Fluazaiadolizine SC treatment, the mortality rate for *H. sojae* was higher than that of *H. glycines*. Responses of 6 nematicidal compounds to two cyst nematodes (*H. schachtii* and *H. trifolii*) were tested using eggs and larvae. Fluopyram SC, Fosthiazate SL and Imicyafos SL showed a high rate of hatching inhibition of 99% or more for both cyst nematodes. Therefore, it can be concluded that these nematicides can readily be used for control of these two cyst nematodes, and there is no difference in nematicidal efficacy between cyst nematode species with respect to the two tested nematicides.

## MANAGING SCN GENETIC RESISTANCE AS WELL AS OTHER CULTURAL PRACTICES TO MAXIMIZE YIELDS AND AVOID RACE SHIFTS IN NATIVE POPULATIONS. Levene, Brian, Quintanilla, M. Michigan State University, 288 Farm Lane, East Lansing, MI.

Many "wild-type" or Plant Introduction (PI) lines of soybeans have been identified which have resistance to soybean cyst nematode (SCN) reproduction. However, there are currently only two or three main sources for genetic resistance to SCN - PI 88788, PI 548402 (Peking), and PI 437654 (Hartwig and CystX \*). The PI 88788 source of resistance is used in over 90% of current SCN-resistant varieties sold in the US. Only a small number of commercial varieties currently use the PI 548402 (Peking) source, and even fewer use the PI 437654 source. Within some soybean maturity groups, there may only be PI88788 resistance available. However, much like herbicide resistance by various weeds, nematodes can also adapt and overcome a single form of management used against them. Developing new multifaceted strategies for SCN management may allow the PI 88788 genetics to continue to be a valuable tool against SCN into the future. Strategies that allow time for additional forms of genetic resistance to be bred into locally adapted soybean varieties to minimize future yield losses to SCN as well as avoidance of alternate SCN hosts in rotation will be of great importance when developing new management practices. Legumes such as peas and lupins, dry beans, and even tobacco have been found to be alternate hosts for SCN. Weeds such as common chickweed (Stellaria media), common mullein (Verbascum thapsus), and hairy vetch (Vicia villosa) have been shown to maintain or increase populations even when soybeans are not present. However, common grass crops such as corn, wheat, oats, other small grains, and forage grasses do not support SCN development. Therefore, growing a non-host crop for one or more years between soybean crops can be an effective form of SCN management. During the spring of 2017, a field in central Michigan where SCN were known to exist and PI 88788 resistant varieties had been commonly been used was selected as a trial site to look at the impact of the most common options for genetic resistance to SCN. Three varieties known to have very similar genetics except for the presence of PI 88788, PI 548402 (Peking), or no resistance to SCN were selected for a multi-year trial. Each variety will be grown continuously in the same plots for five years or planted in rotation with other varieties to determine the impact on yields as well as potential shifts in the race of SCN with each strategy. Additionally, the impact of ILevo seed treatment on SCN development was included in this study. While no significant yield differences were noticed among the three types of SCN resistance in the initial year of this trial, there were differences among them in the second season (2018) for yield as well as nematode development. Preliminary race testing was also completed following the 2018 season to determine the potential shifts in the natural populations of SCN.

NEMATODE MANAGEMENT IN FLORIDA POTATO AND SWEET POTATO USING NON-FUMIGANT NEMATICIDES. **Chang** Liu<sup>1</sup>, Z.J. Grabau<sup>1</sup>, and P.A. Navia Gine<sup>2</sup>. <sup>1</sup>Entomology and Nematology Department, University of Florida, Gainesville, FL 32611. <sup>2</sup>AD-AMA Agricultural Solutions.

Sting nematode (Belonolaimus longicaudatus), stubby-root nematode (Trichodorus and Paratrichodorus sp.), and root-knot nematode (Meloidogyne sp.) are the most important nematode species affecting potatoes and sweet potatoes in Florida. Two field trials were conducted at Hastings, Florida to test the efficacy of fluensulfone (Nimitz), oxamyl (Vydate C-LV), and 1,3-Dichloropropene (1,3-D, Telone II) at controlling sting and stubby-root nematodes on potatoes. One trial was conducted at Live Oak, Florida to test the efficacy of fluazaindolizine (Salibro), fluopyram (Velum Prime), Vydate, and Telone for managing root-knot nematodes on sweet potato. The first trial at Hastings (Trial A) had four treatments: 1) Nimitz 2.5 pt/A, 2) Nimitz 5 pt/A, 3) Telone II 6.5 gal/A, and 4) untreated control. The second potato trial (Trial B) had five treatments: 1) Nimitz 2.5 pt/A, 2) Nimitz 5 pt/A, 3) Nimitz 2.5 pt/A + Vydate 1 qt/A, 4) Telone II 6.5 gal/A, and 5) untreated control. Telone and Nimitz were applied 23 days before planting, and Vydate 45 days after planting (DAP). Soil samples were taken 30 days before planting as well as 55 and 85 DAP. In Trial A, at 55 DAP, both Nimitz at 5 pt/A and Telone decreased sting nematode significantly (P<0.05) with 67% and 86% reduction respectively. At harvest, all nematicide treatments decreased (P<0.05) sting nematode abundances compared to control. In Trial B, at 55 DAP, all nematicide treatments decreased sting nematode count significantly compared to control (P<0.05). No treatment differences were found on nematode counts at harvest. Results indicate that both Nimitz and Telone may be beneficial for managing sting nematode in potato. For the sweet potato trial, six treatments were applied: 1) Salibro 30.7 oz/A, 2) Salibro 61.4 oz/A, 3) Salibro 30.7 oz/A + Vydate L 128 oz/A, 4) Telone II 8 gal/A, 5) Velum Prime 6.8 oz/A, and 6) Vydate L 128 oz/A. Telone was applied 31 days before planting, and all other nematicides were applied 10 days before planting. Soil samples were taken at 64 and 106 DAP. Tubers were dug, sorted into marketable and unmarketable categories and weighed at 126 DAP. At harvest, root-knot nematode abundances were lesser for treatments with Salibro. Telone II had significantly greater (P<0.05) marketable tuber yield than any treatments except for Salibro at 61.4 oz/A. Results suggest that Salibro may control root-knot nematode effectively on sweet potato.

# CONSERVED TOXIN-BASED HOST-KILLING STRATEGY IN ENTOMOPATHOGENIC NEMATODES OF THE *STEINERNEMA* GENUS. Lu, Dihong<sup>1</sup>, D. Chang<sup>1</sup>, L. Serra<sup>2</sup>, A. Mortazavi<sup>2</sup>, and A. Dillman<sup>1</sup>. <sup>1</sup>Department of Nematology, University of California, Riverside, CA 92521. <sup>2</sup>Department of Developmental and Cell Biology, Center for Complex Biological Systems, University of California, Irvine, CA 92697.

It is commonly believed that entomopathogenic nematodes (EPNs) rely on their symbiotic bacteria to kill insect hosts. However, there were also evidence showing that the infective juveniles of *Steinernema carpocapsae* and *S. feltiae* without their symbionts can also kill

insects and reproduce in the cadaver. We hypothesized that the infective juveniles of *Steinernema* spp. not only serve as the vector of their symbiotic bacteria but also actively contribute to host-killing by secreting toxins. We investigated the insecticidal activities of the excreted/ secreted products (ESPs), especially proteins, of four species: *S. carpocapsae*, *S. feltiae*, *S. scapterisci*, *and S. glaseri* which are the most wide-ly used EPNs for biocontrol of insect pests. In our study, millions of infective juveniles of these species were activated by exposure to insect extracts for harvesting ESPs. We used single-nematode RNA sequencing method to compare the transcriptomes of infective juveniles that are activated *in vitro* or *in vivo* and confirmed that the *in vitro* activation of infective juveniles actually mimics the *in vivo* infection process. We tested the ESP activity by injecting 20 nanograms of ESP proteins into fruit flies and found that the flies were killed within a few hours. Using proteomics methods, we analyzed the protein compositions of *S. carpocapsae* and *S. feltiae* ESPs. By separating the ESP proteins into many fractions using fast protein liquid chromatography and by testing insecticidal activity in fruit flies, we identified a few toxic fractions for further analysis of their insecticidal activities. In conclusion, we have found that EPNs in the *Steinernema* genus have a conserved host-killing strategy by releasing toxic protein mixtures. Our research could lead to the discovery of nematode-derived insecticidal toxins that may be used in pest control in agriculture in the future.

## THE USE OF NEW CHEMISTRIES FOR POTATO NEMATODE MANAGEMENT PROGRAMS IN IDAHO, USA. Luff, Kelly<sup>1</sup> and S. L. Hafez<sup>2</sup>. <sup>1</sup>Bayer Crop Science, 3554 East 4000 North, Kimberly, ID 83341. <sup>2</sup>29603 U of I Lane, Parma, ID 83660.

What a potato grower can't see at planting can be painfully visible at harvest. Root-knot nematodes can cause tuber imperfections which impact profit potential. Root lesion nematodes open the door to disease, particularly *Verticillium dahliae*, the causal agent of potato early dying which ultimately results in smaller tubers and reduced yield. Once growers know what they are up against, the next question is which nematicide options offer the safest (environmental & personal), efficacious and economically efficient treatment. Growers should consider and put into practice several control options, including fumigants, nematicides, crop rotation and bio fumigants. New non-fumigant nematicides are being developed by multiple basic manufactures of crop protection chemistries. In general, these new chemistries have lower mammalian toxicity and a safer environmental profile as compared to established nematicides. However, length of activity is shorter and these new compounds must be incorporated into nematode management programs that integrate multiple nematicides and sequential applications. Research with Velum<sup>®</sup> Prime (fluopyram) and Movento<sup>®</sup> HL (spirotetramat) has shown these two chemistries to be effective in managing both root-knot and lesion nematode species in potatoes. Both Velum<sup>®</sup> Prime and Movento<sup>®</sup> HL can be effectively utilized in nematode management programs with Telone<sup>®</sup> II, Vapam<sup>®</sup>, Mocap<sup>®</sup> and Vydate<sup>®</sup>.

TWO NEMATOPHAGOUS *PLEUROTUS* MUSHROOM SPECIES DIFFERENTIALLY CONSUME SOME OF THIRTEEN BACTERI-AL-FEEDING NEMATODE SPECIES BUT ARE THEMSELVES CONSUMED BY OTHERS. **Marlin, Maria<sup>1</sup>, A. Wolf<sup>1</sup>, M. Alomran<sup>2,3</sup>, L. Carta<sup>4</sup> and G. Newcombe<sup>1</sup>.** <sup>1</sup>College of Natural Resources, Department of Forest, Rangeland, and Fire Sciences, University of Idaho, Moscow, ID 83844, USA. <sup>2</sup>College of Agricultural and Life Sciences, Department of Plant Sciences, University of Idaho, Moscow, ID 83844, USA. <sup>3</sup>College of Science, Department of Biology, Princess Nourah bint Abdulrahman University, Riyadh 11671, Saudi Arabia. <sup>4</sup>Mycology and Nematology Genetic Diversity and Biology Laboratory, USDA-ARS, Beltsville, MD 20705, USA.

*Pleurotus* fungi (oyster mushrooms) are said to be nematophagous because they paralyze and consume various types of nematodes. It has never been clear whether that means all nematodes. Here we tested two toxin-producing isolates of *Pleurotus pulmonarius* and *Pleurotus ostreatus* with thirteen bacterial-feeding nematode species: seven of family Rhabditidae, three of Cephalobidae (one with three populations), two of Panagrolaimidae, and one of Diplogastridae.

Nematodes interacted with fungi on water agar until they either consumed and reproduced on *Pleurotus*, or succumbed to its toxin and were consumed. Of the thirteen species, nine were susceptible to *P. pulmonarius* (all individuals were paralyzed) but four (four populations of two cephalobid species, one rhabditid, and one panagrolaimid) survived exposure to *P. pulmonarius*. The resistant four species not only survived but multiplied their numbers by consuming *P. pulmonarius*. A similar trend was observed with nematodes interacting with *P. ostreatus*; however, six species were resistant to *P. ostreatus*. Interestingly, four of these six species were susceptible to *P. pulmonarius*, and interactions overall were differential. *Pleurotus* species are nematophagous toward some nematodes but are also consumed by others in three of the four families assayed. Species-specific interactions point to the need for studies of the host ranges of both "nematophagous" fungi and "fungivorous" nematodes, especially if they are to be used for biological control.

#### INCIDENCE, ABUNDANCE, AND DISTRIBUTION OF PLANT-PARASITIC NEMATODES IN VEGETABLE SYSTEMS OF SOUTH-ERN GEORGIA. Marquez, Josiah and A. Hajihassani. Department of Plant Pathology, University of Georgia, Tifton, GA, 31794.

Plant-parasitic nematodes (PPN) greatly affect vegetable crops throughout the USA. The vegetable industry has been growing in the state of Georgia, yet no study has assessed the incidence, abundance, and distribution of PPN on vegetable crops. Therefore 437 vegetable fields in 30 counties of the vegetable-producing regions of southern Georgia were sampled once during the spring, fall or winter cropping seasons of 2018. A range of 30-40 soil cores were collected every 3-4 m in a diagonal transect through each field. Nematodes were extracted by sieving followed by centrifugal sugar flotation, counted, and identified to genus based on morphological features. The 10 genera of PPN detected were root- knot (*Meloidogyne* spp.), stubby-root (*Paratrichodorus* spp.), ring (*Mesocriconema* spp.), spiral (*Helicotylenchus* spp.), root-lesion (*Pratylenchus* spp.), lance (*Hoplolaimus* spp.), reniform (*Rotylenchulus* spp.), cyst (*Heterodera* spp.), stunt (*Tylenchorhynchus* spp.), and dagger (*Xiphenema* spp.) nematodes. Root-knot nematode (RKN) had greater ( $P \le 0.05$ ) in the fields of eggplant (*Solanum melongena*), tomato (*Solanum lycopersicum*), pepper (*Capsicum annuum*), bean (*Phaseolus vulgaris*), cucumber (*Cucumis sativus*), and cantaloupe (*Cucumis melo* var. *cantalupensis*) than in fields of watermelon (*Citrullus lanatus*) or onion (*Allium cepa*). Higher abundance of RKN are distributed in the southernmost counties. Stubby-root nematodes had the second highest incidence at 49.4% in our survey and

it has been observed to be a potential problem on numerous vegetable crops. Although RKN is the most yield-limiting nematode in the vegetable fields sampled, other commonly occurring PPN including *Mesocriconema* spp., *Heliocotylenchus* spp., and *Pratylenchus* spp., with respective incidence rates of 39.6%, 31.6%, and 20.1%, may also affect vegetable production and warrant further investigation.

MOLECULAR IDENTIFICATION OF *MELOIDOGYNE* SPP. ASSOCIATED WITH VEGETABLE CROPS IN SOUTHERN GEORGIA. **Marquez, Josiah<sup>1</sup>, W. Ye<sup>2</sup>, A. Skantar<sup>3</sup> and A. Hajihassani<sup>1</sup>.** <sup>1</sup>Department of Plant Pathology, University of Georgia, Tifton, GA, 31794. <sup>2</sup>Nematode Assay Section, Agronomic Division, North Carolina Department of Agriculture & Consumer Services, Raleigh, NC 27607. <sup>3</sup>Mycology and Nematology Genetic Diversity and Biology Laboratory, USDA, ARS, Beltsville, MD 20705.

The identification of root-knot nematodes (Meloidogyne spp.) infecting vegetable crops is paramount in Georgia, USA as recent threats of new aggressive Meloidogyne spp. have been reported in neighboring states of Florida (M. enterolobii, M. floridensis, and M. haplanaria), Alabama (M. haplanaria), South Carolina and North Carolina (M. enterolobii). In addition, no molecular approaches have been utilized to discriminate different Meloidogyne species in vegetable production systems of Georgia. Therefore, the aim of this study was to assess the occurrence and incidence of Meoidogyne spp. in vegetable fields of Georgia. Soil samples were collected from 294 Meloidogyne-infested fields in the spring and fall of 2018 throughout the vegetable-producing regions of southern Georgia. For culturing Meloidogyne nematodes, the infested soil samples were potted into a 15-cm-diameter polyethylene pots and tomato (Solanum lycopersicum) cv Rutgers was transplanted. After the tomato was grown for 8 to 10 weeks, adult female nematodes were teased out of the roots for genomic DNA extraction. Each culture had 3 biological replicates for the DNA analyses. Each DNA sample consisted of a single adult female identified using species-specific primers and DNA sequencing of the region in the mitochondrial genome cytochrome c oxidase subunit II (COII) and 18S and 28S rDNA genes. Out of the 211 fields positive with species-specific primers, 69.8% were infested with M. incognita, 27.2% with M. arenaria, 2.9% with M. javanica, and no positive results were found for M. hapla and M. enterolobii. About 31.0% of the fields had mixed populations of M. incognita and M. arenaria followed by M. incognita and M. javanica (3.4%), and all three species (1.5%). Two new species for the state of Georgia, M. floridensis and M. haplanaria, have been identified through DNA sequencing of the COII gene. As expected, M. incognita was the most dominant species followed by M. arenaria. The high rate of mixed populations of M. incognita and M. arenaria could be explained by the region's long history of cotton and peanut cultivation.

#### PLANT PARASITES IN THE WILD: COMMUNITY ANALYSIS OF CRICONEMATIDAE NEMATODES IN GREAT SMOKY MOUN-TAINS NATIONAL PARK. **Matczyszyn, Julianne, T.O. Powers and S.E. Everhart.** University of Nebraska-Lincoln, Department of Plant Pathology, 406 Plant Sciences Hall, Lincoln, NE 68583-0722.

Use of nematodes as bioindicators of soil ecological structure, function, and the role disturbance influences the soil is well documented, but plant parasitic nematodes are the least understood. Plant parasitic nematodes in the family Criconematidae are well-characterized as agricultural plant parasites. However, studies of native habitats show greater diversity and distribution patterns. A limit in knowledge of plant parasitic nematode community diversity restricts the understanding of effects of anthropogenic disturbance above ground on the recolonization of nematodes below ground. Additional information on plant parasites at the community level is needed to fill this gap. Soil core samples were taken and bulked from within 19 All Taxa Biodiversity Inventory (ATBI) 1×1-ha replicated field sites in Great Smoky Mountains National Park. Criconematidae nematodes were extracted using sucrose centrifugation, photographed, smashed, and PCR amplified at the cytochrome oxidase subunit 1 gene and sequenced. Phylogenetic analysis was used to identify nematodes to species and haplotype group. Ecological data for each ATBI site were obtained from public sources and included: elevation, dominant tree species, and disturbance regime. Community data were analyzed using a non- metric multidimensional scaling and did not identify structure at this scale (P > 0.05). Pairwise genetic similarity between individuals was estimated using Jukes-Cantor distance and more detailed analysis using 363 plant species constancy values (>20%), 35 soil characteristics, and five environmental variables (elevation, minimum and maximum temperature, average annual precipitation) at each ATBI site was performed using distance-based redundancy analysis (dbRDA). Results indicated seven variables (4 plant species, 2 soil variables, and 1 environmental variable) predicted 12.52% of the total variation ( $adjR^2 = 0.07$ ; F = 2.21; P = 0.001). Six of the surface texture categories were identified as predictors. A discriminant analysis of principal components was applied to surface texture, which retained 15 principal components and 4 discriminant functions (PC1 = 51.66 eigenvalue, PC2 = 15.75 eignevalue). Analysis of molecular variance indicated surface texture was significant source of population structure (Phi = 0.06; P = 0.001). Collectively, results indicate multiple properties of the environment are influential in Criconematidae nematode distribution and population structure, including soil texture, and plant host constancy and distribution of influential species. Knowledge of ecological patterns of plant parasitic nematodes and their association in native habitats aids in the application of nematodes as bioindicators for the soil ecological structure, function, and response to disturbance.

## PHYLOGENETIC AND POPULATION STRUCTURE OF *MESOCRICONEMA XENOPLAX* ACROSS THE UNITED STATES. **Matczyszyn, Julianne, S. Everhart, T. Harris, K. Powers and T. Powers.** University of Nebraska Lincoln, Department of Plant Pathology, 406 Plant Sciences Hall, Lincoln, NE 68583-0722.

Molecular genetic characterization is an important tool to determine if a single morphological species contains hidden diversity or represents a cryptic species. Previous studies suggest that the plant parasitic nematode *Mesocriconema xenoplax* is such a species. Application of integrated species delimitation analyses, we investigated the species boundaries of *M. xenoplax* collected from 174 sites across the United States. Each site was surveyed using soil cores sampled within a  $40 \times 40$ -m grid and yielded a total of 132 nematodes identified as *M. xenoplax*. Each nematode was measured, photographed, and PCR-amplified at the cytochrome oxidase subunit 1 gene, and sequenced. Phylogenetic analysis was used to identify nematodes to species and haplotype groups (HGs). Sequence data were used to perform seven species delimitation analyses, including: TCS, ABGD, K/ $\Theta$ , and species delimitation plug-in statistics through Geneious. Morphological data were tested using a discriminant function analysis to identify morphological distinction. Spatial genetic structure was analyzed using

a Mantel test to identify isolation by distance. Ecological data for each site were obtained from public sources and included: annual average minimum and maximum temperature, annual average precipitation, associated land cover, elevation, ecoregion, and biome. A pairwise Jukes-Cantor genetic similarity matrix between individuals was calculated and associations with ecological data were analyzed using a distance-based redundancy analysis. Phylogenetic results supported seven HGs ( $\geq$  99/0.99 posterior probability/bootstrap values). Species delimitation, morphological, and spatial genetic structure results further supported two metapopulations, HG 14 and the combining three HGs (HGs 11,12,13 combined). Three variables (land cover, ecoregion, and maximum temperature) predicted 53.61% of the total variation ( $adjR^2 = 0.68.17$ ; F = 3.99; P < 0.01). Metapopulations by land cover showed *M. xenoplax* in herbaceous and woody wetlands were significantly different (AMOVA  $\phi = 0.75$ ; P < 0.01). Results provide empirical evidence for both molecular and ecological differentiation within *M. xenoplax sensu latu* to suggest that this species is indeed a collection of cryptic species.

## HOST STATUS OF YELLOW AND PURPLE NUTSEDGE TO *BELONOLAIMUS LONGICAUDATUS*. Mendes, Maria L., D. W. Dickson, and W. T. Crow. Entomology and Nematology Department, University of Florida, Gainesville, FL 32611.

Belonolaimus longicaudatus, the sting nematode, is among the most important nematode pests of many crops and turfgrasses in Florida, reducing the ability of plant roots to uptake water and nutrients from the soil. Yellow (Cyperus esculentus) and purple (C. rotundus) nutsedges are considered the most difficult-to-manage weed in Florida agriculture and infest many of the same fields as sting nematode. If these nutsedges are hosts to sting nematode they could reduce the effectiveness of nematode management strategies. However, the host status of these important weeds to sting nematode is unknown. With this in consideration, the objective of this study was to determine the host suitability of yellow and purple nutsedges to B. longicaudatus. The experiment was carried out under greenhouse conditions, in a completely randomized design with five replications of three treatments. St. Augustinegrass (Stenotaphrum secundatum) was used as known host to B. longicaudatus for comparison. Clay pots containing 1,400 cm<sup>3</sup> of sand were planted with 6 tubers of either yellow or purple nutsedge, or sprigs of St. Augustinegrass, and later inoculated with 120 B. longicaudatus per pot. After 180 days the nematode population density was assessed along with fresh root and tuber weights. The nematodes were extracted from 200  $\text{cm}^3$  of soil using the sugar flotation method. Roots and tubers were incubated in a mist chamber for 72 hours to determine if the nematodes infected plant tissue. No nematodes were recovered from either roots and tubers of yellow and purple nutsedge or St. Augustinegrass roots. There were no differences (P > 0.05) in the number of *B. longicaudatus* recovered from the three hosts. Both yellow and purple nutsedge were good hosts for B. longicaudatus. The reproduction factor (RF = Pf/Pi) was 1.90 on yellow nutsedge, 3.1 on purple nutsedge and 3.2 on St. Augustinegrass. This data indicates that yellow and purple nutsedges can serve as alternate hosts for B. longicaudatus in agricultural fields and should be considered a factor in sting nematode management strategies.

## ROLE OF CIRCADIAN AND DIURNAL RHYTHMS ON ROOT-KNOT NEMATODE PARASITISM. Mishra, Shova<sup>1</sup> and P.M. Di-Gennaro<sup>1</sup>. <sup>1</sup>Department of Entomology and Nematology, University of Florida, Gainesville, FL 32611.

Root-knot nematodes (RKN; *Meloidogyne* spp.) are obligate plant parasites with a wide host range, infecting over 2,000 plant species. This diverse host range suggests that RKN utilize basal plant biology to communicate with their hosts to establish and maintain parasitism. Light is one such fundamental regulator of plant biology that directs many metabolic, chemical and physiological processes. As these plant processes follow tightly regulated circadian and diurnal patterns, it is likely that obligate plant parasites, like RKN, use similar cues to regulate aspects of the plant-nematode interaction. To investigate the possible influence of host circadian and diurnal rhythms on RKN biology, we conducted a time course experiment in different stages of nematode development. We examined nematode penetration into host roots to see if temporal variations exist during this stage of infection. *Medicago truncatula* seedlings were inoculated with *M. incognita* under 16hr light/8 hr dark conditions. At 24-hours post inoculation, roots were stained for nematode penetration. *M. incognita* penetration was significantly higher when inoculated during the dark, suggesting nematodes are more active at night. To determine if this phenomenon is due to nematode perception of light in the soil, or due to different plant cues, we evaluated RKN egg hatch under constant light and constant dark conditions. Over a 68 hours period, we found no difference in percent of eggs hatched between consecutive time points under both conditions suggesting that hatching was not influenced by time of day. However, eggs incubated in constant light exhibited delayed hatching compared to those in constant dark suggesting light directly influences nematode biology. Understanding the role and origin of circadian and diurnal rhythms in the plant-nematode interaction will likely underscore the importance of exploiting basal plant biology to develop novel control methods for these pathogens.

## TRANSCRIPTIONAL PROFILING OF ROOT-KNOT NEMATODE FEEDING SITES OVER A 24 HOUR PERIOD. Mishra, Shova<sup>1</sup>, and P. M. DiGennaro<sup>1</sup>. <sup>1</sup>Department of Entomology and Nematology, University of Florida, Gainesville, FL 32611.

Root-knot nematodes (RKN; *Meloidogyne* spp.), an obligate plant-parasite, constantly communicates with their host to maintain specialized feeding sites developed from plant cells. RKN likely regulate their interaction by monitoring host biology and host behavior. As plant gene expression varies temporally, especially over a 24hour period, RKN gene transcription might follow similar patterns. We utilized a publicly available RNASeq dataset to examine RKN transcripts over a period of 24 hours and identified approximately 1,500 differentially expressed genes (DEGs) in nematode and model host *Medicago truncatula*. DEGs were minimally binned into 10 expression profiles. Interestingly, more than 80% of nematode DEGs occurred at middle of night compared to middle of the day. Differentially expressed genes may suggest pathways that are central to compatible RKN-host interaction and nutrient allocation in the plant. DEGs were assigned to known Gene Ontology categories to predict their functional roles and associated biological processes revealing plant genes involved in defense response pathway and nematode genes involved in establishing infection are highly upregulated at night compared to day suggesting for strong host-nematode interaction occurring at night. To better characterize the pathways involved in compatible RKNhost interaction we are interested in finding distinct coexpression patterns between nematode and plant DEGs. This study elucidated some pathways that are involved in nematode-host interaction and will reveal some potential plant pathways that could be targeted to develop a management strategy via tolerance or resistance. CHARACTERIZATION OF NATIVE ENTOMOPTHOGEN NEMATODES FOR CONTROL OF *AEGORHINUS SUPERCILIOSUS* (COLEOPTERA: CURCULIONIDAE) ACCORDING THEIR ABILITY TO PERCEIVE VOLATILES RELEASED BY DAMAGED BLUEBERRY ROOTS. **Navarro, Patricia<sup>1</sup>, R.A. Palma<sup>1</sup>, J.A. Urzua<sup>2</sup>, R. E. Ceballos<sup>2</sup>.** <sup>1</sup>Insect Science Laboratory, Agricultural Research Center (INIA), Camino Cajon Vilcun, Temuco, Chile. <sup>2</sup>Insect Chemical Ecology, INIA, Avenida Vicente Mendez # 515, Chillan.

Two native species of entomopathogenic nematodes (EPNs), *Steinernema australe* and *Steinernema feltiae* QU-31, were evaluated for control of *Aegorhinus superciliosus* (Coleoptera: Curculionidae), the most important pest affecting blueberry crops in Chile. Larvae of this curculionidae are located inside the root making galleries and killing the plant. After decades of failed strategies (insecticides and entomopathogenic fungi) for control of these larvae, the use of EPNs has been the most promising tool. The efficacy of the native EPNs (*S. australe* and *S. feltiae* isolate QU-31) was compared with an introduced and currently commercialized *Steinernema feltiae* under laboratory conditions. We hypothesized that native EPNs would be significantly more responsive to volatile signals emitted by blueberry roots damaged by *Aegorhinus* larvae than introduced EPNs. To determine the volatiles produced by damaged blueberry roots, samples were analyzed with SPME and headspace chromatographic techniques. Results indicated qualitative and quantitative differences in the type and amount of the identified compounds between the roots of healthy and infested blueberry plants. The herbivory-induced root volatiles in blueberry plants mainly corresponded to terpenic, aliphatic alcohols, and methyl-salicylate derivative compounds. Under laboratory conditions, the intrinsic efficacy of the native *S. feltiae* QU-31 showed 55% mortality using a dose of 30 IJ/ml five days after application, when compared with the introduced *S. feltiae* showing 23% mortality. Low infectivity (32%) was observed for *S. australe* but a good seeking behavior. Future assays using the volatiles identified to attract the EPNs will be conducted.

ATTENUATED TOTAL REFLECTANCE-FOURIER TRANSFORM INFRARED SPECTROSCOPY AND HYPERSPECTRAL IMAG-ING TECHNIQUES POTENTIAL IN QUALITY CONTROL OF FORMULATED ENTOMOPATHOGENIC NEMATODES. **Nicholas, Kagimu<sup>1,2,3</sup>, and A. P. Malan<sup>1</sup>**. <sup>1</sup>Department of Conservation Ecology and Entomology, Faculty of AgriSciences, Stellenbosch University, Private Bag X1,Matieland 7602. <sup>2</sup>Department of Zoology and Entomology, University of Pretoria, Pretoria 0002, South Africa. <sup>3</sup>Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria 0002, South Africa.

Quality control is very important in upholding standards, during the manufacturing of products according to specifications, including the commercialisation of entomopathogenic nematodes (EPNs). For nematode quality, virulence remains the most important standard for measurement, which is often determined through using either one-on-one, or sand-well, bioassays, which are costly in terms of laboratory consumables and time. Such determination calls for the use of quick, non-destructive and effective quality control techniques, which could include the application of attenuated total reflectance (ATR), in conjunction with Fourier transform infrared spectroscopy (FTIR) and hyperspectral imaging (HSI) tools, with a proven wide application in other fields of research. This study investigated the potential for the quality control of formulated *Steinernema jeffreyense* and *S. yirgalemense* in diatomaceous earth (DE), and the characterisation of different species using ATR-FTIR and HSI. Results report, for the first time, the use of ATR-FTIR spectral analysis in detecting chemometric changes in the formulated EPN product and changes occurring over time, during storage. The changes are mainly for reasons of nematode survival, in response to environmental stresses. HSI was able to differentiate between variables, in terms of differences in nematode densities, in the formulated sample. For EPN characterisation, the study reports close similarities among the species, as detected by the ATR-FTIR.

# USING HIGH RESOLUTION AERIAL IMAGING TO ASSESS NEMATODE AND DISEASE INCIDENCE, SEVERITY, AND CROP IMPACTS IN FLORIDA STRAWBERRY. **Noling, Joseph<sup>1</sup>, J.S. Baggio<sup>2</sup>, and N.A. Peres<sup>2</sup>**. University of Florida, IFAS. <sup>1</sup>Citrus Research & Education Center, Lake Alfred, FL 33850, <sup>2</sup>Gulf Coast Research and Education Center, Wimauma, FL 33598.

In Florida, nematodes and soilborne fungal diseases such as *Colletotrichum, Phytophthora*, and *Macrophomina*, and the Sting nematode, *Belonolaimus longicaudatus*, are very important yield limiting pests of strawberry. For these studies, digital color imaging and in-field assessments of plant size were used to characterize the distribution and degrees of plant stunting, strawberry yield, and within row measures of green plant canopy cover associated with the sting nematode. Disease incidence, severity, and crop impacts were assessed from ground survey and analysis of aerial imagery. Aerial imaging surveys of over 20 commercial field locations were seasonally conducted from November 2017 to March 2018 and Nov 2018 to March 2019, using a DJI<sup>™</sup> Phantom 4 Pro UAS drone equipped with a DJI 24mm 20MP camera with an Exmor R CMOS sensor. Image orthomosaics were created using DroneDeploy<sup>™</sup> cloud software platform with an image resolution of 10 to 20 mm per pixel. Processed RGB and NDVI maps were oftentimes both visually evaluated and impacted plants enumerated and or analyzed using ESRI<sup>™</sup> ArcGIS v10.33. Strawberry canopy cover, relative yields and enumerations of disease incidence and plant stunting were derived from inspection of drone imagesand then compared using regression analysis with ground truth field surveys. It will be demonstrated that these new aerial imaging techniques and greeenness analysis have great potential to facilitate and increase accuracy and precision in quantifying nematode and plant disease incidence, plant growth response, performance of fumigant and nonfumigant pest management practices, and of long term impacts within the strawberry cropping system.

# EVALUATION OF FUMIGANT AND NON-FUMIGANT NEMATODE MANAGEMENT STRATEGIES IN FLORIDA STRAWBERRY 2018-2019. Noling, Joseph<sup>1</sup> and J. Desaeger <sup>2</sup>. <sup>1</sup>University of Florida, IFAS, Citrus Research & Education Center, Lake Alfred, FL 33850. <sup>2</sup>Gulf Coast Research and Education Center, Wimauma, FL 33598.

Because of changing regulatory requirements and the uncertainties of continued availability of soil fumigants, Florida strawberry growers identified the current project of farming without fumigants as an important research priority in 2018. The large scale field trial consisted of sixteen treatments with four replications. The fumigant treatments included deep shank applications of 1, 3-dichloropropene (Telone II<sup>™</sup>; 112 L/ha) with or without Telone C35 (281 L/ha), or PicClor 80 (231 L/ha) applied in-the-bed. The objective was also to evaluate an increased use rate of Chloropicrin to manage *Macrophomina phaseolina* (Charcoal rot) disease incidence and severity. Other fumigant treatments evaluated included bed shank and drip treatments of Paladin\* Pic (79/21%) (374 L/ha), with or without deep shank Paladin\* (140 l/ha)

and drip applied metam potassium (KPAM\*; 580 L/ha). In general all drip applied nonfumigants nematicides, including preplant and postplant applications of Nimitz\* (4.1 l/ha), Velum\* (0.5 l/ha), Majestene\* (18.7 l/ha), Dazitol\* (58.5 l/ha) and Nemakill\* (3.5 l/ha) were metered into the irrigation water over a 1 to 3 hour injection period. A drip applied fungicide (Ridomil\*) and a bed-sprayed herbicide (Goal\* 2XL 2 pt/a) was also included to expand the spectrum of pest control activity of the nonfumigant system. For all treatments, a highly gas retentive totally impermeable Berry Plastics Total Blockade TIF, 1.25 mil was installed immediately after treatment application and or bed formation. Strawberry canopy convergence, expressed as the rate of foliar growth both within and between planted rows of strawberry, was significantly delayed season long, particularly early within the non-treated controls and all of the nonfumigant treatments. Weed control and disease incidence, severity, and plant mortality were significantly higher for both preplant and late season plant density assessments within the untreated controls and all nonfumigant treatment combinations. Compared to untreated controls, the nonfumigant treatment combinations generally increased strawberry yields by 5.2%, while yields were increased by 16.5 to 27.8 % dependent upon the particular fumigant combination utilized. Strawberry yields were depressed up to 22% in the untreated controls and nonfumigant treatment combinations due to increased disease incidence and plant mortality and overall smaller plant sizes. The smaller plant sizes also resulted in significantly lower relative yield and significantly lower total canopy greenness quantitatively assessed from green pixel counts from aerial images. It would appear that plant growth and productivity was proportional to the level of the most limiting pest control strategies, most notably those treatments lacking strong fungicidal and herbicidal activity. Many questions remain concerning how to best use these new nonfumigant nematicidal products, with and without supplemental fumigant or other IPM strategies which will be needed to expand the breadth of pest control activity.

EXPESSION OF A NEMATODE PARASITISM GENE IN ARABIDOPSISENHANCES PLANT TOLERANCE TO SALT. **Noureddine Hamamouch**<sup>1,2</sup>, **C. Li<sup>1</sup>, and E. L. Davis<sup>1</sup>.** <sup>1</sup> Dept. of Entomology and Plant Pathology, North Carolina State University, Raleigh, NC. <sup>2</sup>Laboratory of Biotechnology and Sustainable Development of Natural Resources, Polydisciplinary Faculty, Univ. Sultan Moulay Slimane, Beni Mellal, Morocco.Parasitism genes expressed in the esophageal gland cells of cyst nematodes encode secreted effector proteins that alter plant cells to promote parasitism. Expression of the *Heterodera schachtii 20E03* parasitism gene in transgenic *Arabidopsis thaliana* plants was investigated for potential effects on plant phenotype. There were no visible changes in the root phenotype of transgenic Arabidopsis lines that constitutively expressed *Hs20E03*, yet roots were more susceptible to cyst nematode infection. Interestingly, expression of *Hs20E03* increased plant tolerance to salt stress; transgenic *Hs20E03* plants were able to germinate and grow in the presence of high concentrations of NaCl (180mM). In addition, expression of double stranded RNA (dsRNA) complementary in sequence to the *Hs20E03* transcript in transformed *A. thaliana* was used to silence the *20E03* transcript in *H. schachtii* that penetrated plant roots to analyze for the potential effects on nematode parasitism of the host roots. A significant reduction in the number of cysts was observed in Arabidopsis lines that expressed dsRNA of *20E03* when compared to control roots. These findings indicate, that the cyst nematode 20E03 effector promotes plant parasitism by the nematode, potentially by modifying cellular salt stress tolerance during the infection process.

IMPROVING ENTOMOPATHOGENIC MOVEMENT AND INFECTIVITY WITH PHEROMONE EXTRACTS. **Oliveira-Hofman, Camila<sup>1</sup>, F. Kaplan<sup>2</sup>, G. Stevens<sup>3</sup>, E. Lewis<sup>3</sup>, S. Wu<sup>1</sup>, H.T. Alborn<sup>4</sup>, A. Perret-Gentil<sup>2</sup> and D. Shapiro-Ilan<sup>1</sup>. <sup>1</sup>USDA-ARS, SEFTNRL, Byron, GA 31008, USA <sup>2</sup>Pheronym, Inc., Gainesville, FL 32601, USA. <sup>3</sup>Department of Entomology, Plant Pathology and Nematology, University of Idaho, Moscow, ID 83844, USA. <sup>4</sup>USDA-ARS Center for Medical, Agricultural and Veterinary Entomology, Gainesville, FL 32608, United States.** 

Infectivity and dispersal of entomopathogenic nematodes (EPNs) are key factors in the biocontrol potential of EPN species. Previous studies demonstrated that extracts from EPN-infected hosts enhance infective juvenile dispersal and efficacy. Here we explored the potential to use the specific pheromones isolated from infected hosts to enhance EPN dispersal and insect pest suppression. The first objective of this study was to determine the impact of ascaroside pheromones on the movement of infective juveniles (IJs) in soil columns baited with Tenebrio molitor larvae. We found that pheromones induced higher numbers of Steinernema carpocapsae and Steinernema feltiae to move towards T. molitor larvae in the bottom of the column in comparison to nematodes treated with infected cadaver macerate and control treatments. For S. carpocapsae the number of IJs that infected T. molitor larvae was also superior for the pheromone treatment, while for S. feltiae pheromone and macerate treated IJs infected T. molitor at the same rate. The second objective was to look at pecan weevil and black soldier fly larvae survival in the greenhouse due to EPNs treated with or without pheromones. For both S. carpocapsae and S. feltiae, pheromone treated nematodes of action, eppendorf tube tests with T. molitor showed that infectivity, measured as invasion rate, of S. carpocapsae and S. feltiae was also greater in pheromone treatments in comparison to control treatments. This novel research demonstrates pheromone-mediated behaviour manipulation of a biological control agent to enhance pest control potential.

## MANAGING PLANT-PARASITIC NEMATODES IN SUB-SAHARAN AFRICA WITH BANANA PAPER: "WRAP AND PLANT", A NOV-EL CONCEPT. **Opperman, Charles<sup>1</sup>, A. Affokpon<sup>2</sup>, L. Cortada<sup>3</sup>, D. Coyne<sup>3</sup>, R. Guenther<sup>1</sup>, S. Koenning<sup>1</sup>, R. Mathew<sup>1</sup>, T. Sit<sup>1</sup>, M. Byrd<sup>1</sup>, T. Pirzada<sup>1</sup>, L. Pal<sup>1</sup>, E. Davis<sup>1</sup>, S. Khan<sup>1</sup>. <sup>1</sup>NC State University, Raleigh, NC. <sup>2</sup>Universite Abomey-Calavi, Benin. <sup>3</sup>IITA, Nairobi, Kenya.**

Smallholder farming practices in sub-Saharan Africa (SSA) include continuous cultivation (often monoculture) with limited inputs, and virtually non-existent seed (piece) treatment techniques. Yam and potato are primary examples and are extremely important and valuable crops for smallholder farmers in SSA. In many cases, greater than 50% of the crop is lost to plant parasitic nematodes (PPN), primarily due to a lack of available, affordable control options. We developed an innovative, cost effective "Wrap and Plant" (W&P) methodology for crop protection from PPN. "W&P" technology is an innovative concept based on banana fibre paper, as a mechanism to deliver micro-doses of the nematicide abamectin to the root zone – presenting an economically viable option for SSA growers, including smallholder farms. Importantly the incorporation of active ingredients into a lignocellulose matrix, such as banana paper, enables effective distribution of crop protection agents without interfering in smallholder farming practices. Our multi-year replicated field trial results demonstrate the efficacy and affordability of "W&P" technology for management of PPNs on yam and white potato crops. On-farm field trials were conducted in 3 yam-growing agro-ecological zones in Benin from 2015-17. Tuber yield and quality were consistently and substantially greater than conventional farmers' practices across all on-farm sites each year, and farmers indicated that "W&P" treatment resulted in larger, longer, and cleaner tubers. Further, food quality and preparation of yam flour revealed a strong preference for the "W&P"-treated yams. Application of "W&P" technology resulted in significant reductions in final nematode populations compared to farmers' practice. We observed an 80% reduction in final yam nematode (*Scutellonema bradys*) populations in tuber peels. Not only was this largely responsible for the high tuber quality, but also indicates substantially reduced risks of post-harvest tuber damage and loss due to this nematode. In addition, final root-knot nematode (RKN) populations were 80% lower compared to farmer practice, resulting in healthier plants and tubers. On-farm field trials were performed in 2 white potato-growing agro-ecological zones in Kenya during 2016-17. Significantly, these trials were conducted in fields infested with potato cyst nematode (PCN), the most important nematode pest of potato in Kenya and worldwide. Potato yield was observed to be 3- fold greater across all on-farm sites when treated with "W&P" compared to conventional practices, and outperformed all other treatments by a substantial margin. Potato yields were consistently fourfold higher (26.41 T/ha) for PCN field trials and 20.24 T/ha for RKN field trials, than yields observed under farmers' practices (5.67 T/ha). W&P technology also provided the highest profitability for farmers, compared with other treatments. This novel and innovative technique provides a simple and effective option for nematode management in yam and potatoes, in addition to numerous other crops.

#### ROOT EXUDATES CONVEY HOST-SPECIFIC MESSAGES THAT CONTROL THE SHORT-RANGE UNDERGROUND ORIENTA-TION OF PLANT PARASITIC NEMATODES. Sang-Wook Park, Wenshan Liu, Alexis L. Jones, Heather N. Gosse and Kathy S. Lawrence. Department of Entomology and Plant Pathology Auburn University, Auburn, AL 36849, USA.

Plant parasitic nematodes (PPN) are microscopic roundworms that develop an obligate parasitic relationship with hosts, causing cell and tissue mortality. These damages have become of great agronomic importance resulting in an annual loss of ~14 % world crop production. However, our knowledge regarding the pathobiology of PPN is still rudimentary. We hence have revisited present working models and information gaps within, in order to revamp our understanding of plant and PPN interactions. It has long been proposed that chemotaxis is a primary mean by which PPN locate host roots, but the identity and the mode of action of chemoattractants that deliver 'host-specific message' to PPN are largely elusive. We have thus developed a unique multidimensional agar-based motility assay to assess the short-range motility and orientation of three PPN (i.e., *Rotylenchulus reniformis, Meloidogyne incognita* and *Heterodera glycines*) towards root exudates of their respective host and nonhost crops (i.e., cotton, peanut and soybean). As predicted, *R. reniformis* and *M. incognita* were attracted to root exudates of cotton and soybean (hosts), but not to the exudates of peanut (nonhost).

Likewise, *H. glycines* was attracted to soybean (host) root exudates, together underpinning the intrinsic role of root exudates in conveying the host specificity of PPN. Moreover, PPN selectively identified and targeted to hydrophilic, but not hydrophobic, fractions of root exudates, supporting that groundwater should be the effective matrix for chemotaxis associated with PPN and their host plant interactions. Moreover, we have employed a confocal laser-scanning microscope and captured real-time 'live' images between *R. refniormis* and cotton roots (i.e., resistance, tolerance and susceptible phenotypes) to investigate if cottons activate hypersensitive response to limit the establishment and spread of PPN infections. However, cotton roots did not develop localized necrosis at the sites of PPN infections. Instead, we observed that a tolerance line has significant larger numbers of root hairs, comparing to a resistance and a susceptible line, suggesting the potential roles and importance of root growth and/or hair developments in plant tolerance against PPN infections. Moving forward, we have employed a system biology approach to **a**) discern tolerance-associated genes and **b**) determine if those genes are involved in root morphology, by analyzing differential transcriptomes between tolerant and susceptible cotton lines before and after PPN infections.

### STEINERNEMA FELTIAE SHOWS A BEHAVIORAL SHIFT TO NON-DISPRSAL IN THE ABSENCE OF PHEROMONE SIGNALS. Perret-Gentil, Abigail<sup>1</sup>, D. Shapiro-Ilan<sup>2</sup>, J. Sun<sup>1</sup>, A. Mirti<sup>1,4</sup>, E. Sampson<sup>1,4</sup>, K. C. Schiller<sup>1</sup>, E. E. Lewis<sup>3</sup>, F. Kaplan<sup>1</sup>. <sup>1</sup>Pheronym, Inc., Davis, CA 95618. <sup>2</sup>USDA-ARS, Southeast Area Fruit and Tree Nut Research, Byron, GA 31008. <sup>3</sup>University of Idaho, Dept. of Entomology, Plant pathology and Nematology, Moscow, ID83844-2329. <sup>4</sup>Current address: University of Florida, Gainesville, FL 32610.

Emergence, dispersal, and foraging are key behaviors for entomopathogenic nematode (EPN) infective juveniles (IJs) to find insect hosts. A mixture of ascaroside pheromones regulates emergence of *Steinernema feltiae* IJs from consumed insect host cadavers. Once the IJs emerge and move away from the cadavers in the soil, they are no longer exposed to pheromones from the host cadaver and search for new hosts in the absence of pheromone signals. It is unknown how the absence of pheromone signals affects the behavior of EPNs. This study elucidates how *S. feltiae* IJs' dispersal behavior changes in the absence of pheromone signals. The *S. feltiae* IJ dispersal time course in the absence of pheromone signal revealed that populations of IJs underwent a behavioral shift from dispersal (60-80% dispersing) to non-dispersal (0-20% dispersing) within 4-12 days at room temperature (20°C). Furthermore, this behavioral shift to non- dispersal was accelerated by 2 days at high (30°C) and low (15°C) temperatures. The behavioral shift is important for nematode dispersal since a lower dispersal mode conserves energy for IJs and increases survival while waiting for a new food source. After the behavioral shift, when the IJs were exposed to pheromones from host cadavers, IJs were stimulated to disperse. Next, we tested whether pheromone signal had an effective period by monitoring the dispersal after exposing populations of IJs to pheromone signal, suggesting that there was an effective period. These results provide a strong foundation for connecting the behavioral studies of pheromone signals to uncovering molecular mechanisms of parasitism and pathogenicity. Furthermore, it gives insight into how the pheromone signals can be used to extend the shelf life and to improve the efficacy of EPNs and other biocontrol organisms for successful eco-friendly agriculture.

BIODIVERSITY OF *THELASTOMA* SPP. IN NORTH AMERICAN MILLIPEDES AND CONTINUED EFFORTS TOWARD REVISION OF THE GENUS. **Phillips, Gary and E. C. Bernard.** Entomology & Plant Pathology, University of Tennessee, 2505 E. J. Chapman Drive, Plant Biotechnology Building, Room 110, Knoxville, TN 37996.

The geographical distribution of thelastomatid nematodes that parasitize arthropods in North America is poorly known. Worldwide, nematodes within the superfamily Thelastomatoidea are found in a wide range of definitive arthropod hosts, including cockroaches, beetles, mole crickets, scorpions and millipedes. Among the Thelastoma spp. found in North American millipedes, only five species have been recognized: T. attenuatum (type species), T. collare, T. krausi, T. labiatum, and T. spicatum. Thelastomatid nematodes can be separated from rhigonematids, ransomnematids, travassosinematids and rhabditids by the shape of the long esophagus, basal bulb with grinding valve, absence of a pre- anal sucker, a single spicule or none in the male, both sexes typically with a long tail, and usually eight apparent cephalic papillae. Field collections between 2013-2019 produced many more species than the five that are currently described from North America. Nearly 1,280 millipedes spanning 5 orders, 19 families, and 61 species from 21 states were collected, identified and dissected. A total of 11,152 nematodes belonging to the genus Thelastoma were extracted, preserved, and identified. Thelastoma spp. were collected from the diplopod orders Polydesmida, Spirobolida and Spirostreptida, of which representative specimens were identified from seven millipede families: Abacionidae, Choctellidae, Euryuridae, Rhinocricidae, Spirobolidae, Spirostreptidae, and Xystodesmidae. Among these seven families, 40 species of millipedes were identified as viable hosts. Thelastoma loads were more abundant in the millipede hindgut (72.9%) than in the midgut (27.1%). Females were much more prevalent than males (10:1), adults slightly outnumbered juveniles (1.3:1) and there were more than twice the number of juveniles located in the hindgut when compared to the midgut. The mean number of Thelastoma spp. nematodes extracted from the intestines of the millipedes was 9.8 (range 0- 768). Morphological characterizations were conducted with bright field, differential contrast, phase contrast and scanning electron microscopies; molecular work was accomplished utilizing SSU (18S) and LSU (28S) rDNA to inferrelationships. Currently, our work has identified at least 10 new North American Thelastoma spp. This work focuses on the beginning stages of revising the genus Thelastoma. Classification within Thelastoma has been problematic and revisionary studies are underway to determine relationships between the 82 described species, including the 25 species specific to millipedes.

## BEST MANAGEMENT PRACTICES FOR ROOT KNOT NEMATODE (*MELOIDOGYNE HAPLA*) IN DAYLILY (*HEMEROCALLIS* SPP.) PRODUCTION. Kristin Poley and Marisol Quintanilla.

Bare-root production of Hemerocallis, daylilies, is of major economic importance among Michigan's ornamental industry. Production of clean nursery plant material can be a challenge for bare-root ornamental crops grown under field conditions due to the occurrence of plant-parasitic nematodes. Root knot nematodes (Meloidogyne spp.) in particular are an important challenge to Hemerocallis production. A replicated field trial was established in 2018 in Zeeland, Michigan with the objective to establish effective management strategies during field production of Hemerocallis in each year of development (three years total), by examining alternatives to fumigation in an effort to reduce nematode populations. One field was selected for preparation of first-year Hemerocallis planting. Prior to planting, the chosen field was divided into plots that were either fumigated with Telone or not fumigated. First-year daylily plants, variety "Going Bananas", were planted into both the fumigated and non-fumigated portions of the field. Each treatment was replicated six times and were compared to the same variety of daylilies planted in the adjacent fumigated plot. The following treatments were implemented: an untreated control, three different compost blends, four bio-nematicides, two chemical nematicides, a chemical nematicide root dip, and soil steaming: a non-fumigant soil sterilization technique using boiling water. An initial soil sample was collected on the same day as treatment application and daylily planting (May) for use as a baseline for nematode populations within each plot. Soil and root samples were taken from each treatment at a mid-season sampling point (July), and at the end of the season (October). Soil samples were composite samples (i.e. ten subsamples that are homogenized). Fields were sampled in a zigzag pattern to reduce variability. Root samples were inspected for galls. Initial nematode counts from the soil are complete, but the remaining samples are pending. Once nematodes are extracted, plant- parasites (with a special emphasis on Northern root knot nematode) and beneficial nematodes will be identified and quantified. In addition, soil health parameters including beneficial nematode diversity, soil organic matter, and plant yield will be compared per treatment. Indemnify (Fluopyram) as a soil drench and as a root dip performed better than all other treatments across all plant vigor measurements including the number of plant eyes, plant height, and root weight, but was not always statistically greater. The top four treatments for controlling root knot nematodes in the first year of growth were soil steaming, fumigation, one chemical nematicide (TerraClean [Hydrogen Dioxide]) and one bio-nematicide (Azaguard [Azadirachtin]). Each of these treatments had significantly fewer root knot nematodes than the control and one of the compost blends, but were not significantly different from the remaining treatments. All treatments will be re- applied and evaluated in each of the next two years (2019 and 2020) to the same plots in order to evaluate best management practices for root knot nematode throughout the entire field cycle of Hemerocallis.

PATTERNSOFSOIL COMMUNITIES IN GLACIAL RETREAT AREAS USING META-ANALYSIS. **Pothula, Satyendra K.<sup>1</sup>, D.H. Wall<sup>2</sup>, W. B. Lyons<sup>3</sup>, M. Diaz<sup>3</sup>, N. Fierer<sup>4</sup>, I. D. Hogg<sup>5</sup>, N. Dragone<sup>4</sup>, N. P. Lemoine<sup>6</sup>, B. J. Adams<sup>1</sup>. <sup>1</sup>Department of Biology, Brigham Young University, Provo, UT 84602 USA. <sup>2</sup> Department of Biology, Colorado State University, Fort Collins, CO 80523 USA. <sup>3</sup> School of Earth Sciences, Ohio State University, Columbus, OH 43210 USA. <sup>4</sup> Department of Ecology and Evolutionary Biology, University of Colorado, Boulder, CO 80302 USA. <sup>5</sup> Polar Knowledge Canada, Canadian High Arctic Research Station, Cambridge, Bay, X0B 0C0 Nunavut Canada. <sup>6</sup> Biological Sciences, Marquette University, Milwaukee, WI 53233 USA.** 

Glacier forefields are the newly exposed landscapes formed by retreating glaciers. As glaciers retreat, their forefields provide a unique opportunity to study the order and timing of biotic colonization, and how this influences the structure of successive ecological communities. In the last century glaciers across most of the cryosphere have receded at an unprecedented pace. On the other hand, for millennia the Transantarctic Mountains and Dry Valleys of Antarctica have been some of the most stable surfaces on the planet, providing an optimal model for studying how soil ecosystems responded to gradual deglaciation since the last glacial maximum. However, even these areas are beginning to experience regional warming due to global effects of climate change. One of the strongest drivers of ecosystem change is increased biological connectivity (dispersal and gene flow), largely due to increased hydrological activity and katabatic winds. We have conducted preliminary studies in the Transantarctic Mountains on how soil communities have responded to deglaciation in the past (since the Pleistocene), but there have also been many published studies from other parts of the world looking at how soil ecosystems are responding to rapid,

contemporary deglaciation events. Using the information from these published studies, our objective was to use a meta-analysis approach to test whether there are any common, predictable patterns of community assembly in response to glacial recession. To collect relevant data from published studies we used the Web of Science indexing service and the Google Scholar database using different combinations of 68 search terms. A total of 157 unique articles were found using these search terms. Of these, articles containing soil biotic richness and abundance data from glacial retreat areas that were suitable for informing our hypothesis were included in our analysis. The results indicated that Actinobacteria and Acidobacteria were consistently abundant along the chronosequence. However, the proportion of Proteobacteria and Bacteriodetes were dominant in soils that had been exposed for less than 90 years and gradually decreased in older soils. Analysis of fungal communities revealed that members of Basidiomycota followed by Ascomycota were predominant throughout the chronosequence. Among nematode trophic groups, bacterial feeders dominated soils from 0 to 80 years since deglaciation but start to decline after 50 years. In contrast, plant feeding nematodes gradually increased and were dominant in soils that were exposed for more than 80 years since glacial recession. Predatory nematodes were completely absent in soils that had been deglaciated for more than 5000 years. The results of our analysis reveal replicated patterns of phylogenetic and functional diversity associated with post-glacial-recession community assembly. From these patterns we infer fundamental processes responsible for shaping them, including strong environmental/geochemical filters, and biotic properties, such as dispersal kernels and biotic interactions. Thus, our work leverages an understanding of how past and present soil ecosystems are responding to deglaciation to inform predictions of how contemporary soil ecosystems will respond to future en

POTENTIAL OF NICOTINAMIDE ADENINE DINUCLEOTIDE (NAD) FOR MANAGEMENT OF ROOT-KNOT NEMATODE IN TOMATO. **Regmi, Homan<sup>1</sup>, N. Abdelsamad<sup>2</sup>, C. Khanal<sup>1</sup>, P. DiGennaro<sup>3</sup> and J. Desaeger<sup>1</sup>. <sup>1</sup>Entomology and Nematology Department, University of Florida, Gulf Coast Research and Education Center (UF, GCREC), Florida. <sup>2</sup>United States Department of Agriculture-Agriculture Research Services (USDA-ARS), San Joaquin Valley Agricultural Sciences Center, Parlier, CA. <sup>3</sup>Entomology and Nematology Department, UF, Florida.** 

Triggering innate plant immunity using chemical elicitors is an attractive strategy to combat plant pathogens. Nicotinamide adenine dinucleotide (NAD) is one such chemical elicitor that regulates plant defense responses to different biotic stresses. We have demonstrated NAD induces defense response pathways, reducing root-knot nematode (*Meloidogyne hapla*) penetration and increasing plant mass in tomato (Abdelsamad et. al., 2019). We wanted to further evaluate if NAD is effective against other root-knot nematode species, and its potential to treat and prime tomato seedlings in transplant trays, prior to planting in the field. Here, we report the effects of NAD application on one of the most important root-knot nematodes affecting tomato in Florida *M. incognita*, using a popular commercial tomato in Florida, HM1823. Three different NAD concentrations (1mM, 0.1mM and 0.01mM) and three application timings (pre; post; pre and post inoculation) were tested. NAD significantly reduced J2 penetration, gall number, and increased plant vigor at 1mM, 30 days after transplanting (DAT). We conducted two greenhouse trials to test the longer-term impact of 1mM NAD (pre, pre + post or post applications) on season-long growth and yield of tomato. In both trials, the pre + post 1mM NAD application was able to reduce gall severity by ~10% until the end of the crop season (105 DAT) but there was no significant difference in plant biomass and fruit yield among the treatments. Our future testing will evaluate the long- term effect of higher NAD concentrations on gall severity and growth and yield of tomato.

### NEMATICIDE APPLICATION THROUGH DRIP IRRIGATION SYSTEMS FOR SOUTHERN ROOT-KNOT NEMATODE MANAGE-MENT. Nunes Rondon, Marina, B. Lawaju, W. Groover, D. Dyer, K. Gattoni, W. Sanchez, K.S. Lawrence. Entomology and Plant Pathology Department, Auburn University, AL 36849.

The southern root-knot nematode (RKN, Meloidogyne incognita) is a major problem in the southern U.S., causing severe yield losses in fruits, vegetables, and row crops. In this research, different nematicide combinations were drip applied to evaluate RKN management on pepper ('PS 09942815', Capsicum annuum) and squash ('Prelude II', Cucurbita pepo). Test plots were located at the Auburn University Brewton Agricultural Research Unit in Brewton, Alabama. Squash seeds were directly sown in the field on 01 June 2018, while six weekold pepper seedlings were transplanted to the field on 08 June 2018. Plots consisted of single-rows that were 0.8 m wide with 7.6 m long beds covered with white plastic mulch. Tests were arranged in a RCBD with six replications. Combinations of Salibro (fluazaindolizine), Vydate L (oxamyl), and Velum Prime (flyopyram) were applied at planting, 14 days after planting (DAP) and 21 DAP, through drip irrigation system. Untreated plots were included as the control. Plant height (pepper), vine length (squash), biomass, and nematode population density were collected at 32 DAP. Nematodes were extracted by soaking the roots in a 6% NaOCl solution on an orbital shaker for 4 minutes and the nematodes collected on a 25-µm sieve. Nematode population density was recorded as total number of RKN eggs per gram of root. Plots were harvested six times from 40-63 DAP for squashes and five times from 52-103 DAP for peppers. Marketable squashes and peppers were used to determine the total yield. Data were analyzed with SAS 9.4 using PROC GLIMMIX, and means compared using Tukey-Kramer method ( $P \le 0.1$ ). All nematicide combinations, except the higher rate of Salibro, exhibited greater plant height than the untreated control ( $P \le 0.1$ ). The vine length of squash plants did not exhibit the same response, where all treatments were the same as the untreated control. Pepper and squash biomass were not affected by the nematicide applications ( $P \le 0.1$ ). RKN numbers per gram of root in the squash trial were higher for the untreated controls ( $P \le 0.1$ ). Different nematicide combinations numerically increased the marketable yield of pepper and squash, although no significant differences were observed ( $P \le 0.1$ ). On pepper, two applications of Vydate L increased the yield by 10.4%, and two applications of Salibro increased the yield by 9.2%. Yield response on squash was increased by 39.2% using two applications of Salibro, and 25.1% when the higher rate of Salibro was applied. Overall, the results indicated that applications of Salibro and Vydate nematicides through drip irrigation systems could be a strategy to manage RKN in vegetable crops in Alabama.

LOCALIZATION OF VIRAL AND HOST RNA WITHIN SOYBEAN CYST NEMATODE VIA WHOLE-MOUNT FLUORESCENCE *IN SITU* HYBRIDIZATION. **Ruark-Seward, Casey L., E.L. Davis, and T.L. Sit**. Department of Entomology and Plant Pathology, North Carolina State University, Raleigh, North Carolina 27606.

Nematode-infecting RNA viruses have recently been discovered via transcriptome sequencing. In soybean cyst nematode (SCN; *Heterodera glycines*), seven single-stranded RNA viruses have been identified from transcriptome data and experimentally confirmed with qRT-PCR and Sanger sequencing. Presently, there is still much unknown about the relationship between these viruses and the nematode host. In this study, we localize three viruses within the soybean cyst nematode: SCN socyvirus-1 (SbCNV-1), SCN nyami-like virus (NLV), and SCN bunya-like virus (BLV). To visually locate the viruses, whole-mount fluorescence *in situ* hybridization (FISH) methodology was developed for SCN pre- parasitic second-stage juveniles (ppJ2s). Two SCN populations with differing viral titers (LY1 and MM21) were used as a comparison for viral probe fluorescence intensity. Viral RNAs for all three viruses were abundant in cells throughout the SCN ppJ2 body of the high titer (LY1) population but absent within the majority of the intestinal tract. A significant reduction in viral fluorescence intensity was observed in a similar body pattern in ppJ2 of the low-titer (MM21) SCN, highlighting the specificity of the FISH method. As controls, viral RNAs were colocalized with host mRNA glyceraldehyde 3-phosphate dehydrogenase (GAPDH) for full body localization and a (4G06) secretory ubiquitin protein expressed specifically within the subventral glands. In addition, viral replication was confirmed in SCN eggs and ppJ2s via qRT-PCR detection of the anti-genomic RNA strands.

## DAMAGE POTENTIAL OF *PRATYLENCHUS PENETRANS* ON SOYBEAN. Saikai, Kanan, and A. E. MacGuidwin. Plant Pathology Dept., University of Wisconsin-Madison, WI 53706.

Pratylenchus penetrans, one of the most damaging species of the genus with a wide host range, is often detected in soybean fields in Wisconsin. Our objective was to describe the effect of P. penetrans on soybean yield and plant growth under field and controlled conditions. The field experiment was conducted on irrigated loamy sand for two years at a research station in Hancock, Wisconsin. Two-meter one-row plots were established at the emergence (VE) stage of soybean at 87 and 114 randomly-spaced points in 2017 and 2018, respectively. Soil samples were collected between soybean rows spaced 75 cm apart at VE for an estimate of the initial population density (Pi). Nematodes in soil and dead root fragments were assayed using centrifugation/flotation and incubation assays, respectively. Soybean plants were harvested at final maturity by hand. The mean Pi was 201 (3 to 695) in 2017 and 165 (0 to 1313) in 2018. The relationship of Pi with soybean yield was linear with each nematode contributing 0.018% (P = 0.01) to the reduction in seed yield. The model suggested a 3 to 3.6% average yield loss for the two site-years. Some plots with low Pi showed seed yield as low as seed yields at plots with higher Pi. In order to reflect this, we conducted a logistic regression for the relationship between the probability of yield loss and Pi. Five percent yield loss was considered given that \$23 per acre would be lost to the nematode damage based on the gross value of soybean production in 2018 at state average. Higher probability of the 5% yield loss was associated with the increase of Pi (P = 0.007). The probability of 5% yield loss was 0.44 and 0.46, and the relative risk of having yield loss was 1.28 to 1.35 times greater to the fields without the nematode at average Pi in 2017 and 2018, respectively. The nematode also inhibited plant growth at V2 growth stage and reduced pod number and seed density at harvest (P<0.05). Three green-house experiments using seven doses of P. penetrans inoculum ranging from 0 to 20000 nematodes per pot confirmed the field experiment. Nematode negatively affected (P < 0.05) plant growth, pod number, and seed yield. Yield reduction by P. penetrans was most likely due to a reduction in pod number. The nematode reduced the ratio of dry weight of seed per plant to the sum of shoot and root dry weights (P = 0.03). Given P. penetrans is an obligate parasite, it may have an important role in manipulating the energy partitioning of plants, favoring plant growth over reproduction.

## EFFICACY OF ENTOMOPATHOGENIC NEMATODES ON SMALL HIVE BEETLES (*AETHINA TUMIDA*) IN KALMIA LOAMY SAND. Sanchez, WinDi, G. Williams, and K. Lawrence. Department of Entomology and Plant Pathology, 209 Rouse Life Science Building, Auburn University, Auburn, AL 36849.

Small hive beetles (SHB), Aethina Tumida, are an insect pest of honey bees that destroy comb and weaken colonies. The main objective of this research is to fill in the gap between controlled laboratory conditions and field trials for entomopathogenic control of SHB in order to improve current Integrated Pest Management practices. The research objective for this project was to determine the effect of natural verses sterilized soil and population density of SHB larva on successful pupation. Soil used for this study was Kalmia loamy sand (80% sand, 10% silt, 10% clay), which is inherent to the SE region of the United States. The six population densities of SHB were 0, 5, 10, 20, 40, and 80 SHB larva per 60mm x 15mm petri dish containing 130g natural or sterilized Kalmia loamy sand. Sterilized soil was autoclaved at 121° C for 60 minutes three times with 24 hours between each sterilization. Petri dishes were placed in a factorial arrangement of a RCBD, with soil condition (sterilized or natural) as the main factor and population density as the second factor and replicated 3 times. The petri dishes were placed into a humidity controlled incubator at 25° C, 80% RH, and 0 light for 20 days. Data were analyzed using PROC GLIMMIX and LSMEANS separated by Tukey-Kramer at  $P \ge 0.05$ . The interaction of soil condition and SHB population density was not significant with  $P \ge 0.10$ . Results with Kalmia loamy sand indicate sterilization numerically enhanced SHB pupation by 11%. The lowest population densities of 5 SHB larva had highest percent emergence to adult beetles in both the sterilized and natural soil with 86%. Population densities of 10, 20, 40, and 80 SHB ranged from 66% to 46% successful pupation, decreasing as the beetle larva populations increased. This suggests that petri dishes containing sterilized or natural soil and lower population densities of SHB larva had greater success pupating. Established optimum SHB population densities will then be advanced to the next objective of evaluating efficacy of six entomopathogenic nematodes - two Heterorhabditis spp. and four Steinernema spp. - towards controlling SHB larva and pupa in three Alabama soil types to improve current Integrated Pest Management practices.

INTEGRATED NEMATODE AND SOIL HEALTH MANAGEMENT IN THE WESTERN HIGHLANDS OF GUATEMALAN POTATO PRODUCTION SOILS: II – INTEGRATED EFFICIENCY OF SOIL AMENDMENTS.**Sanchez-Perez, Amilcar<sup>1</sup>, G. I. Alvarez<sup>1</sup>, B. S. Sipes<sup>2</sup>, S. Kakaire<sup>3</sup>, C-L. Lee<sup>4</sup>, A. Sacbaja<sup>1</sup>, C. Chan<sup>2</sup>, and H. Melakeberhan<sup>3</sup>**. <sup>1</sup>Faculty of Agronomy, University of San Carlos, Guatemala City, Guatemala. <sup>2</sup>University of Hawaii at Manoa, Honolulu, HI 96822 USA. <sup>3</sup>Department of Horticulture, and <sup>4</sup>CANR Statistical Consulting, Michigan State University, East Lansing, MI 48824 USA.

As part of the USAID's Horticulture Innovation Laboratory goal to alleviate nutrition and food insecurity, this collaborative research was conducted in smallholder potato production areas in Huehuetenango and Xela in the Highlands of Guatemala. These regions have soil health and plant-parasitic nematode management problems. The Huehuetenango region is at 3,200 m to 3,353 m altitude laying over Mollisol soil group (class) and the Xela region is at 2,896 m over Andisols. An experiment was conducted in each region over two years to answer multiple questions. In each region, the effects of amending soils either with or without bio-mix (BioCopia) and 0, 318, or 454 kg of composted chicken manure at eight locations were tested. The bio-mix consisted of Guatemalan isolates of Purpureum and Bacillus applied at 1.8 kg/m<sup>2</sup> to suppress harmful nematodes. Looking at the relationship between potato cyst nematodes (Globodera spp.) population density (x-axis) and potato yield and/or C:N ratio (y-axis) simultaneously, this presentation reports integrated efficiency of the treatments. The model expresses cyst numbers, C:N ratio, and potato yield data of each treatment as a percent of the untreated control. A regression of x and y determines four best-to-worst case outcomes for managing cysts, yield, soil nutrients and the environment simultaneously. Cysts were extracted from 100 cm3 of soil at-planting, midseason and at-harvest using acetone flotation methods and quantified. Soil C and N were measured. Cyst population density increased in both soils, suggesting that the treatments were not effective. Potato yield increased in Andisols and decreased in Mollisols by about 5%. Organic matter did not change in Andisols, but increased in Mollisols. Cyst population density was very high before the treatments were initiated in both soil groups. A combination of the cyst, C:N ratio and potato yield data suggest that the Andisols are likely to benefit more from soil amendments than the Mollisols, which are high in organic matter. Further studies are needed to explain the underlying differences between the soil groups.

BECOMING AN ENDO-PARASITE: DIGITIZATION AND ARCHIVING THE TEM DATA COLLECTION OF DR. BURTON ENDO. Schroeder, Nathan<sup>1</sup>, D.H. Hall<sup>2</sup>, and L.K. Carta<sup>3</sup>. <sup>1</sup>Dept. of Crop Sciences, University of Illinois, Urbana, IL 61873. <sup>2</sup> Dept. of Neuroscience, Albert Einstein College of Medicine, Bronx, NY. <sup>3</sup>USDA-ARS, Beltsville, MD.

Electron microscopy (EM) has been essential for the description of nematode anatomy. Dr. Burt Endo (1926- 2005), spent the majority of his career with the USDA-ARS conducting ultrastructural investigations on diverse parasitic nematode species leading to almost 100 publications. His data were collected prior to the adoption of digital cameras for EM and large-scale storage of digital image files. The estimated 37,000 glass plate negatives and photographic prints collected by Dr. Endo are a valuable resource for nematologists; however, to date the majority of these data were not easily accessible. Following his retirement, these files were stored in a building that subsequently suffered storm-related damage and was slated for demolition. During the summer of 2018, we salvaged the physical data and available lab notebooks from this building. Most of the data can be traced back to identifiable entries in the notebooks. We have recovered extensive raw data associated with publications. For example, Dr. Endo's sole publication on the animal- parasitic nematode *Onchocerca volvulus* comprised 34 separate EM micrographs, while the archives contain 1,155 prints. We also identified data not associated with any publication. For example, data on *Trichodorus similis* and *Heterodera trifolii* were identified within the collection. We have begun digitizing these data using a high-resolution scanner. Following digitization and annotation, these data will be made publicly available through the online WormAtlas will be written to include descriptions on non-*C. elegans* species.

CAN NEMATICIDE APPLICATION BE REDUCED IN SOD-BASED ROTATION? Schumacher, Lesley<sup>1</sup>, Z.J. Grabau<sup>1</sup>, W.T. Crow<sup>1</sup>, D.L. Wright<sup>2</sup>, I.M. Small<sup>2</sup>, H.L. Liao<sup>2</sup>. <sup>1</sup>Department of Entomology and Nematology, University of Florida, Gainesville, FL, 32611. <sup>2</sup> North Florida Research and Education Center, University of Florida, Quincy, FL 32351.

Reniform nematode (Rotylenchulus reniformis, RN) is a significant pathogen of cotton that is typically managed by crop rotation or nematicide application. In the southeast United States, a conventional crop rotation consists of peanut followed by two years of cotton. An alternative, sod-based rotation uses two years of bahiagrass followed by one year each of peanut and cotton. Because peanut and bahiagrass are poor/non-hosts of RN, sod-based rotation may be an effective way to manage this nematode. It is necessary to develop sustainable farming practices that reduce damage by plant parasites while maintaining populations of beneficial nematodes, such as those that feed upon bacteria and fungi. Therefore, nematode populations were investigated in cotton phases of sod-based and conventional rotations with or without irrigation and nematicide at a long-term research site at the North Florida Research and Education Center. Objectives were to: 1) determine yield benefits of nematicide application for cotton in conventional and sod-based rotations; and 2) evaluate effects of nematicide on nematode populations in conventional and sod-based rotations. The soil nematode community was assessed in spring, summer, and fall in 2017 and 2018. Cotton yield was collected in October 2017 and 2018. RN populations were greater in conventional cotton than sod-based cotton in the fall of 2017 and 2018 (P<0.05). Nematicide application did not have a consistent effect on cotton yield. In 2017, nematicide application increased yield in second-year conventional cotton but did not affect cotton yield in any other rotation phase. However, in 2018, nematicide application decreased yield in sod-based cotton, but did not affect cotton yield in any other rotation phase. Furthermore, nematicide application did not have a consistent effect on bacterivore and fungivore abundance - increasing abundances in sod-based cotton but decreasing them in second-year conventional cotton. Overall, sod-based rotation reduced RN populations. Sodbased rotation had greater cotton yield than the conventional rotations. Nematicide use may not be needed to improve cotton yield in sod-based rotation. Furthermore, beneficial nematodes were not negatively impacted by nematicide application in sod-based rotation.

# RESISTANCE OF SWEETPOTATO GENOTYPES TO MELOIDOGYNE ENTEROLOBII AND ITS DISTRIBUTION IN EASTERN NORTH CAROLINA **Tanner Schwarz<sup>1</sup> and Eric Davis<sup>1</sup>**. <sup>1</sup>Department of Entomology and Plant Pathology, North Carolina State University, Raleigh, NC 27695.

*Meloidogyne enterolobii* is an aggressive root-knot nematode species that has been detected in North Carolina within the last decade. In collaboration with the NCDA Nematode Assay Lab, root-knot nematode samples from sweetpotato, soybean, and tobacco grown in the eastern-half of North Carolina were analyzed using PCR to assay for the presence of *M. enterolobii*. This species has been detected

in Columbus, Sampson, Harnett, Johnston, Wayne, Greene, Wilson, and Nash counties. Three populations of *M. enterolobii* from NC sweetpotato farms, and one population from a NC soybean farm have been cultured on roots of tomato plants. Potential resistance to *M. enterolobii* in sixty selected sweetpotato [*Ipomoea batatas* (L.)Lam.] genotypes was evaluated in greenhouse experiments. 10,000 eggs of *M. enterolobii* were inoculated on each sweetpotato genotype grown in a 3:1 sand to soil mixture. Nine weeks after inoculation, roots were weighed, and nematode eggs were extracted from roots. Genotype susceptibility to *M. enterolobii* was assessed as the number of nematode eggs per gram of root. Susceptible genotypes included Covington averaging 3,730 eggs per gram of root, Beauregard at 4,263 eggs/gram root, NCDM04-001 at 19,613 eggs/gram root, and HiDry at 9,925 eggs/gram root. Resistant sweetpotato genotypes included Tanzania at 1.81 eggs/gram root, Murasaki-29 at 2.98 eggs/gram root, Bwanjule at 5.06 eggs/gram root. Dimbuka-Bukulula at 11.30 eggs/gram root, Jewel at 1.91 eggs/gram root, Centennial at 2.87 eggs/gram root, and Tib-11 with 10.77 eggs/gram root. We are collaborating with NC State sweetpotato breeders to incorporate the observed resistance to *M. enterolobii* into commercial sweetpotato cultivars.

SOIL FREE LIVING NEMATODES – IN AGRICULTURAL SYSTEM AS BIOINDICATORS. **Goldstein Shakartchy E.<sup>1</sup>, G. Eshel<sup>2</sup>, O. Rinot<sup>3</sup> and Y.Steinberger<sup>1</sup>.** <sup>1</sup>The Mina & Everard Goodman Faculty of Life Sciences, Bar-Ilan University, Ramat-Gan 5290002, Israel. <sup>2</sup>Soil Erosion Research Station, Ministry of Agriculture & Rural Development, Rishon-Lezion; POB 30, Beit-Dagan 5020000, Israel. <sup>3</sup>Institute of Soil, Water and Environmental Sciences, Agricultural Research Organization, The Volcani Center, Israel.

Soil bioindicators are organisms that have the capacity to detect changes in natural environment and monitor soil health by being sensitive to changes in chemical, physiological or behavioral, correlated with soil use. The nematodes community are one of those groups that help in detecting the smallest footprint changes on the environment as they: abundant in soil, easy to sample, well classified into functional (feeding) groups and having the ability to respond readily to changes in the soil's physical and chemical properties. In the present study, soil free living nematode community composition, density and diversity were used as tool in order to evaluate alterations between different long term agricultural practices as part of soil health evaluation. Soil samples were collected during the autumn 2016 from two climate zone in the north (Jezreel Valley) and in the south (north- western Negev), Israel. The soil samples were collected under three long-term soil management as following: control (UC), field crops (FC), orchards (OR) and from three soil layer: 0-10 cm, 10-30 cm and 30-60 cm. Chemical analysis (soil moisture, organic matter, pH, C/N, conductivity) and biological analysis were conducted as follows: nematode communities were extracted (using the Bermann funnel procedure), counted using a compound microscope, following molecular analysis for species, trophic group, diversity and indexes determination. Soil moisture was found to be significantly (p<0.05) affected by study site, were the north site was higher in comparison the south site. Moreover, the sampling location at each site was as follow orchard > field crops > control significantly (p < 0.05). They had been found as well affected by the soil layer and location. The soil free living nematodes had been found to follow the changes in soil moisture and organic matter content which are correlated with the long-term management at each site. From all the four soil free living nematodes trophic groups the bacterial feeding (BF) trophic group was found to be the dominant reaching 80 individual /100g dry soil followed by the fungi feeders (FF) (32 individual /100g dry soil), and decrease to plant parasites (PP) (15 individual /100g dry soil) and omnivore predators.(OP) (12 individual /100g dry soil). Based on the above data the calculated indices that had found to explain the treatment 72 effect. The FF / BF ratio was significantly higher (p < 0.05) in orchards, elucidating that a higher BF based food exhibits higher and faster decomposition rates in comparison to a fungi-based webs. Species diversity (H') was found to be significantly higher (p<0.05) in the north compared with the south. No differences were found in the ratio (FF + BF) / PP, the ratio of bacterivores and fungivores to plant parasites. According to the size of the population, the species diversity and division into the trophic groups, the soil free living nematode community was found to a reliable bioindicator for soil health determination.

PREFERENTIAL INFECTIVITY OF ENTOMOPATHOGENIC NEMATODES IN AN ENVENOMED HOST. **Shapiro-Ilan<sup>1</sup>**, **David**, **G.N. Mbata<sup>2</sup>**, **H.T. Alborn<sup>3</sup>**, **and M.R. Strand<sup>4</sup>**. <sup>1</sup>USDA-ARS, 21 Dunbar Road, Byron, GA 31008. <sup>2</sup>Agricultural Research Station, Fort Valley State University, Fort Valley, GA 31030. <sup>3</sup>USDA-ARS CMAVE, Gainesville, FL 32608. <sup>4</sup>Department of Entomology, University of Georgia, Athens, GA 30602.

Entomopathogenic nematodes and parasitoid wasps are biocontrol agents used for management of insect pests such as the Indian meal moth, *Plodia interpunctella. Habrobracon hebetor*, a parasitoid wasp, injects a paralytic venom into *P. interpunctella* larvae before laying eggs. A prior study indicated that the entomopathogenic nematode *Heterorhabditis indica* preferentially infects *P. interpunctella* that have been envenomed by *H. hebetor* and results in the present study found a similar preference by the entomopathogenic nematode, *Steinernema glaseri*. Thus, we investigated four hypotheses to determine why nematode infection rates are higher in envenomed hosts: 1) elevated CO<sub>2</sub> emission from envenomed hosts attracts nematodes, 2) paralysis prevents hosts from escaping nematodes, 3) volatile chemicals emitted from envenomed hosts attract nematodes and enhance infection, and 4) reduced immune defenses in envenomed hosts lead to increased nematode survival. Results indicated that envenomed *P. interpunctella* larvae emitted lower amounts of CO<sub>2</sub>than non-envenomed larvae. Physical immobilization of *P. interpunctella* larvae increased infection rates by *H. indica* but did not increase infection rates by *S. glaseri*. Volatile emissions from envenomed and non-envenomed hosts were collected and analyzed by thermal desorption gas chromatog-raphy/mass spectrometry. The most prominent compound in envenomed hosts, 3-methyl-3-buten-1-ol, was discovered to be an effective cue for *S. glaseri* attraction and infection but was not an effective stimulus for *H. indica*. Envenomed *P. interpunctella* exhibited a stronger immune response toward nematodes than non-envenomed hosts. Overall, we conclude that different mechanisms explain preferential infection in the two nematode species: host immobilization for *H. indica* and chemical cues for *S. glaseri*.

TRICKY PARASITES: HOW NEMATODES OBTAIN THEIR VITAMINS FROM PLANTS! **Siddique S<sup>1,3</sup>**, **Hiltl C, Radakovic ZS<sup>1</sup>**, **Chopra D<sup>1</sup>**, **Holbein J<sup>1</sup>**, **Anjam MS<sup>1</sup>**, **Habash S<sup>1</sup>**, **Riemer E<sup>1</sup>**, **Gioran A<sup>2</sup>**, **Bano D<sup>2</sup>**, **Grundler FMW<sup>1</sup>**. <sup>1</sup>INRES, Molecular Phytomedicine, University of Bonn, Germany. <sup>2</sup>German Center for Neurodegenerative Diseases (DZNE), Bonn, Germany. 3 Department of Entomology and Nematology, University of California Davis, US.

Vitamin B5 (VB5) is an essential nutrient that is synthesized via a three-step process in plants. In Arabidopsis, *AtPANB1* and *AtPANB2* encode the enzyme for the first step and *AtPANC* the enzyme for the last step of the pathway. In comparison to plants, multicellular animals absorb VB5 from their diet. Cyst nematodes are biotrophs, and parasitism is based on the formation of a syncytium in the roots from which nematodes withdraw their nutrients. Here we investigated the role of VB5 during cyst nematode interaction with Arabidopsis. We found that expression of *AtPANB1* and *AtPANB2* is strongly induced upon infection, and this upregulation is essential for nematode development. In comparison to *AtPANB*, *AtPANC* is not upregulated and does not play a role in parasitism. Notably, we identified a cyst nematode PANC gene (*HsPANC*), and showed that the nematodes are able to perform the last step of VB5 biosynthesis using *HsPANC*. A comprehensive, biochemical, molecular, and genetic analysis revealed that compartmentalization of VB5 biosynthesis between plants and nematodes is necessary to avoid feedback/feed-forward inhibition and ensures a continuous supply of VB5 to rapidly developing nematodes.

LAND USE AND CLIMATE VARIABLES INFLUENCE THE STRUCTURE OF NEMATODE COMMUNITIES IN THE CAATINGA DRY FOREST. Silva, Juliane Vanessa Carneiro de Lima<sup>1</sup>, M.N.C. Hirschfeld1, J.E. Cares<sup>2</sup>, and A.M. Esteves<sup>1</sup>. <sup>1</sup>Department of Zoology, Universidade Federal de Pernambuco, Av. Prof. Moraes Rego, 1235 - Cidade Universitária 50670-901, Recife, PE, Brazil. <sup>2</sup>Department of Phytopathology, Universidade de Brasília, Darcy Ribeiro Campus 70910-900, Brasília, DF, Brazil.

Seasonally dry tropical forests have confronted strong environmental impacts due to human activities such as agriculture and ranching, as well as variations in climate conditions. Soil nematodes are sensitive to these changes, given that soil and climate characteristics influence their survival and occurrence. We evaluated the effect of soil, rainfall and temperature on the structure and taxonomic composition of nematode communities in areas with different types of land use (agricultural areas, secondary forest and natural forest) in the Caatinga, at Catimbau National Park, Pernambuco, northeastern Brazil. Specifically, we hypothesized that: (1) the abundance and diversity of soil nematodes would be higher in less-disturbed areas; (2) the nematode communities would be affected by the type of land use and would differ from each other; (3) nematode communities would be structured based on the soil, rainfall and temperature; and (4) the proportion of each trophic group of nematodes would differ among the different types of land use. We recorded a total of 17,177 nematodes belonging to 104 genera. The results showed that nematode abundance and richness were highest in the secondary forest and lowest in the agricultural areas. Additionally, nematode diversity did not differ among types of land- use. However, secondary forest showed the highest levels of diversity, followed by natural forest and agricultural areas. Nematode community composition varied among the types of land use. The genus Acrobeles contributed most to the differences in nematode community structure among the three study areas; its specimens were present in all study plots, although in different levels of abundance. Different soil properties as well as monthly mean rainfall and temperature were strongly related to the differences in composition among the agricultural areas, secondary forest and natural forest, accounting for 65.42% of the total variation. The total abundance of each trophic group was also affected by types of land use. Bacterivores and plant parasites dominated the nematode communities and accounted for 62% and 68% of the nematodes, respectively. In general, our results indicate that agricultural activity in the Caatinga affects the structure of nematode communities, and that soil characteristics and climate variables also strongly affect the structure and composition of these communities.

MOLECULAR AND MORPHOLOGICAL EVIDENCE FOR THE PRESENCE OF ALFALFA CYST NEMATODE, *HETERODERA MED-ICAGINIS*, IN THE UNITED STATES **Skantar, Andrea M.<sup>1</sup>, T. Powers<sup>2</sup>, S. Hafez<sup>3</sup>, Z. Handoo<sup>1</sup>, T. Harris<sup>2</sup>, R. Higgins<sup>2</sup>, P. Mullin<sup>2</sup>, T. Todd<sup>4</sup>, and K. Powers<sup>2</sup>. <sup>1</sup>Mycology and Nematology Genetic Diversity and Biology Laboratory (MNGDBL), USDA, Beltsville, MD. <sup>2</sup>University of Nebraska-Lincoln, Lincoln NE 68583-0722. <sup>3</sup>Department of Plant Pathology, University of Idaho, Boise, ID. <sup>4</sup>Kansas State University, Manhattan Kansas.** 

Specimens of *Heterodera* have been collected from alfalfa fields in Kansas, Montana, and Utah. DNA barcoding with the COI mitochondrial gene indicate that the species is not *Heterodera glycines*, soybean cyst nematode,*H. schachtii*, sugar beet cyst nematode, or *H. trifolii*, clover cyst nematode. Maximum likelihood phylogenetic trees show that the alfalfa specimens form a sister clade most closely related to *H. glycines*, with a 4.7% mean pairwise sequence divergence across the 862 nucleotides of the COI marker. Morphological analyses of juveniles and cysts conform to the measurements of *H. medicaginis*, the alfalfa cyst nematode originally described from the USSR in 1971. Initial host testing demonstrated that the nematode reproduced on alfalfa, but not on soybeans, tomato, or corn. Collectively, the evidence suggests that this finding represents the first record of *H. medicaginis* in North America. Definitive confirmation of this diagnosis would require COI sequence of eastern European isolates of this species. Additional surveys would help further delineate the distribution of this species within the U.S.

### FROM *CAENORHABDITIS* AND *PRISTIONCHUS* TO REAL NEMATODES: THE CHALLENGES FOR MODERN BIOLOGY. **Ralf J. Sommer.** Max-Planck Institute for Developmental Biology Department for Integrative Evolutionary Biology.

Nematology is divided into an applied branch with phylogenetically unrelated parasites and some cherry-picked free-living species that can easily be propagated in the laboratory, becoming important models for various aspects of the modern life sciences. *Caenorhabditis elegans* has championed this process with several discoveries and methodological developments resulting in three Nobel prizes, thereby giving great hope to applied nematology. But where do we stand today and what are the biggest challenges for a modern biology in applied nematology? I will argue that theoretical and experimental studies in evolutionary ecology and comparative genomics, although originally unrelated, point towards the rapidly evolving genome as the largest challenge for applied biology. First, studies in various systems have recently revealed that ecologically relevant traits evolve most rapidly. And parasitism is an ecological trait. Second, comparative genomics in several nematode taxa, some of which include deep taxon sampling of extremely closely related species, have indicated that genomes evolve much more rapidly than previously assumed. This results not only in frequent gene duplication events, but also involves the emergence of novel genes including so-called *de novo* genes. Third, experimental studies in nematodes and insects alike show that the formation of novel morphological and behavioral traits often involve such young genes. I will provide case studies from our own work on self-recognition and comparative genomics in *Pristionchus* nematodes to highlight the importance of novel genes for ecologically relevant traits. I will argue that applied nematology will have to seriously consider the non-conserved parts of the genome to properly understand parasitism.

# POST-INFECTION DEVELOPMENT OF RENIFORM NEMATODE ON RESISTANT SOYBEAN LINES JTN-5203, PI 404166, AND 02011-126-1-5-1-1. Stetina, Salliana R., J. R. Smith, and J. D. Ray. USDA Agricultural Research Service, PO Box 345, Stoneville, MS 38776 USA.

Soybean growers in the southeastern U.S. are faced with the challenge of producing a profitable crop in fields infested with reniform nematode (Rotylenchulus reniformis). The soybean lines JTN-5203, PI 404166, and 02011-126-1-5-1-1 were previously identified as supporting a lower level of root infection by reniform nematode than susceptible soybean lines, but information on post-infection effects on the nematode was lacking. Therefore, the objective of this study was to characterize post-infection development and fecundity in these resistant lines. In three growth chamber experiments using these lines and the susceptible control cultivar Braxton, nematode development early and late in the infection cycle and nematode fecundity were assessed. In each experiment seeds were planted into containers filled with 120 cm<sup>3</sup> of a steam-pasteurized soil mix (1 sandy loam soil:2 sand). Upon stand establishment, 500 reniform nematodes (mixed vermiform life stages) were added to the soil in each container. A completely randomized design with 10 replications was used for each experiment, and experiments were conducted twice. To assess nematode development early in the infection cycle, root infection on 10 plants of each soybean line was measured at 2-day intervals through day10. Assessment of nematode development late in the infection cycle began 15 days after inoculation (DAI) and continued at 5-day intervals through day 30. In both development experiments, root-associated nematodes were classified as either vermiform, swelling (enlargement of body but not yet assuming the kidney-shape characteristic of this species), reniform (kidney-shaped female without egg mass), or gravid (kidney-shaped female with egg mass). Nematode fecundity was measured using plants collected at the 25- and 30-day intervals. Up to 50 arbitrarily selected gravid females were hand- picked from each soybean line, and the number of eggs were counted after crushing the female under a cover slip on a glass slide. There was a slight (1 or 2 day) delay in root infection on the resistant lines as compared to Braxton. Delayed development was noted as early as 10 DAI, with 50% of the population on Braxton classified as gravid while only 20% to 25% of the nematodes in populations developing on the resistant lines had begun laying eggs. The resistant line 02011-126-1-5-1-1 maintained a higher percentage of nematodes in the swelling phase of development late into the infection cycle as compared to JTN-5203 and PI 404166. All three of the resistant soybean lines produced significantly fewer eggs per female compared to Braxton, with a 68 to 95% reduction at 25 DAI, and 80 to 98% reduction at 30 DAI. Thus, it appears that the post-infection mechanisms contributing to resistance in these lines include delayed infection, slower development, and reduced reproduction. The greatly delayed development in 02011-126-1-5-1-1 suggests this line has different or additional mechanisms contributing to reniform nematode resistance as compared to JTN-5203 and PI 404166.

IT TAKES TWO FLINTS TO MAKE A FIRE: UNRAVELLING THE INSECTICIDAL PARTNERSHIP OF *XENORHABDUS BOVIENII* AND *STEINERNEMA* NEMATODES. **Stock, S. Patricia.** School of Animal and Comparative Biomedical Sciences, The University of Arizona. 1117 E. Lowell St, Tucson AZ, 85721.

Fitness of *Steinernema* (Nematoda: Steinernematidae) nematodes is associated to their relationship with their *Xenorhabdus* (Gama-Proteobacteria: Enterobacteriaceae) partners.Upon killing of the insect host, *Xenorhabdus* produce a plethora of secondary metabolites which not only degrade the insect host tissues providing nutrients for the nematodes to grow and reproduce, but also preserve the cadaver from decomposition and attack of soil saprobes, which could eventually interfere with the completion of their life cycle. Studies conducted by my team and others have shown that the level of reliance of *Steinernema* nematodes with their bacterial symbionts is variable, ranging from a tight and obligate relationship to a facultative one. This is dependent on the *Steinernema-Xenorhabdus* partnership involved. Fitness of *Steinernema* IJs can also be affected by their association with non-cognate bacteria. While many *Steinernema* species can produce successful infections in an insect host, when coupled with non-cognate symbiont, their virulence and reproductive fitness is usually reduced. In summary, *Xenorhabdus* are thought to have driven variation in the success of their *Steinernema* hosts. Furthermore, differences in the success of this mutualistic partnership may have contributed to the maintenance of their symbiotic relationship over an evolutionary time scale. In this presentation, I will summarize the research findings of my team in relation to the fitness costs in the *Xenorhabdus bovienii-Steinernema* partnership, based on genomic and transcriptomic data coupled with biochemistry and wet lab bioassays.

## THE POTENTIAL FOR THE MOVEMENT OF NEMATODES ON GOLF SHOES. Stouffer-Hopkins, Anna. 578 Wilson Rd., Rm. 118, East Lansing, MI 48824.

Plant-parasitic nematodes are microscopic organisms that rely on macro fauna, weather events, and water movement to transport them to new locations. The potential movement of nematodes between golf courses on golf shoes was investigated. In collaboration with the MSU men and women's golf teams, shoes were sampled at 4 different time points with 4-8 sets of shoes per team per sampling date. The soil, that adhered to the bottom of golf shoes, was brushed into collection bags; soil collection was done within two days of use. The methods used to process the soil samples varied depending on the amount of soil collected. When less than one gram of soil was collected it was rinsed and poured over a 650-mesh screen. Soil samples between 1 and 10 g of soil were rinsed into a 400ml beaker, stirred, and poured over a 650-mesh screen. Soil samples for two minutes, and poured over a 650-mesh screen. Total nematodes present in each sample were enumerated using light microscopy at X40. Fewer than 5 nematodes were recovered from soil samples that had less than 1g of soil. The 1-10g soil samples contained 2-60 nematodes. Samples with 10g soil and above contained 30 to 530 nematodes. The majority of shoes sampled had less than 1g of soil. When it rained, more soil adhered to the shoes which led to a greater number of nematodes. This survey indicates nematodes are transported by golfers.

## INVESTIGATIONS INTO THE HOST STATUS OF GRAINS AND TURFGRASSES TO THE NEWLY DETECTED *HETERODERA PRATENSIS* IN MICHIGAN. **Stouffer-Hopkins, Anna, F. Warner, J. Vargas.** 578 Wilson Rd., Rm. 118, East Lansing, MI 48824.

In 2017, *Heterodera pratensis* was detected on turfgrass in Michigan. This was the first detection of this nematode in the western hemisphere. The impact of this nematode on its host is unknown, and the host range is not well studied. The host range of *H. pratensis* was investigated; 17 monocots, 7grains<sup>\*</sup>, 9 turfgrasses<sup>\*\*</sup>, and 1 wild grass<sup>+</sup>, were inoculated with eggs and second-stage juveniles then destructively sampled 14 and 35 days post inoculation. Soil was processed using a modified centrifugation flotation method, and nematodes were visualized in the roots using acid fuchsin staining. The nematodes were counted, and the life stage observed. Adults of *H.pratensis* were observed on 13 of 17 monocots tested. This is the first confirmation that *Triticum aestivum*<sup>\*</sup>, (common wheat), *Hordeum vulgare*<sup>\*</sup> (barley), *Secale cereal*<sup>\*</sup> (rye), and *Festuca perennis*(syn. *Lolium multiflorum*)<sup>\*\*</sup> (annual ryegrass), are hosts for *H. pratensis*. *Poa annua*<sup>\*\*</sup> (annual bluegrass), *Agrostis stolonifera*<sup>\*\*</sup> (creeping bentgrass), *Lolium perenne*<sup>\*\*</sup> (perennial rygrass), *P.trivialis*<sup>\*\*</sup> (rough bluegrass), *P.pratensis*<sup>\*\*</sup> (kentucky bluegrass), *A. capillaris*<sup>\*\*</sup> (colonial bentgrass), *A. canina*<sup>\*\*</sup> (velvety bentgrass), and *P.nemoralis*+(wood bluegrass) were also found to support development. *Sorghum bicolor*<sup>\*</sup> (sorghum), *Zea mays*<sup>\*</sup> (corn), *Panicum miliaceum*<sup>\*</sup> (white millet), and *Avena sativa*<sup>\*</sup> (oat), were all found to be non-hosts. The presence of *H. pratensis* in Michigan may have implications for Michigan's small grain industry based on the results of this investigation.

FORENSIC ECOLOGY: A COMPARATIVE ANALYSIS OF NEMATODE SUCCESSION IN SOILS IMPACTED BY ANIMAL AND HU-MAN DECOMPOSITION. **Taylor, L. Stacy<sup>1</sup>, A.R. Mason<sup>1,2</sup>, G. Phillips<sup>3</sup>, E.C. Bernard<sup>3</sup>, J.M. DeBruyn<sup>1</sup>. <sup>1</sup>Department of Biosystems Engineering & Soil Science. <sup>2</sup>Department of Microbiology, and <sup>3</sup>Department of Entomology and Plant Pathology, University of Tennessee, Knoxville, TN 37996.** 

In terrestrial environments, decomposition of vertebrate mortalities results in changes in local chemistry and microbial taxa. Soil microfaunas are also presumably impacted, but have received comparatively little attention. The objectives of these studies were to: 1) assess the use of nematodes to indicate decomposition progress, 2) document nematode successional patterns in animal- and human-impacted soils, and 3) identify potential indicator taxa. Two decomposition experiments were devised; the first used beavers (Castor canadensis) placed in the summer, the second used summer and winter trials of deceased human subjects. All subjects were placed in direct contact with soil and allowed to decompose naturally for a full year. In both experiments soil interface (1-cm deep) and core (15-30-cm) samples were taken to assess nematode population dynamics. Nematodes were identified to family and genus by microscopy. In both experiments, nematode abundance, richness, and diversity responded strongly to the addition of decomposition products; however, the magnitude and timing of the changes differed seasonally in the human study. At the onset of active decay, nematode abundances increased, while richness and diversity decreased in the beaver and human winter studies. In contrast, nematode abundance in the human summer study sharply decreased, remaining low for 50 days with subsequent peak abundances an order of magnitude lower than that observed in the human winter study. In all cases, communities present during early soil enrichment were dominated by B1 enrichment opportunists: Rhabditidae (notably Pelodera and Diploscapter), and Diplogasteridae. In the beaver study, successional patterns emerged by mid- skeletonization (day 110) with enrichment by generalist taxa: Aphelenchoides (F2) and Acrobeloides (B2), followed by Tylencholaimidae (F4), and Prismatolaimus (B3). Filenchus (F2) abundance was depressed below control levels for the duration of both the beaver and the human summer studies. In the human summer study, periodic increases of Aphelenchoides (F2) and Acrobeloides (B2) occurred within a continued strong B1 enrichment response, but successional patterns did not emerge, suggesting that soils were still strongly impacted by decomposition products. The human winter study exhibited similar patterns in early phases and is still ongoing. Our studies strongly suggest that patterns observed in nematode succession under animal analogues may not be applicable for investigation of human decomposition progression, and that seasonal differences have a profound effect upon nematode response to soil enrichment.

SALIBRO<sup>™</sup> (REKLEMEL<sup>™</sup> ACTIVE): A NOVEL NEMATICIDE FOR THE CONTROL OF *MELOIDOGYNE SPP.* IN ROOT VEGETA-BLES IN NORTH AMERICA. **Temple, Joshua<sup>1</sup>, T Thoden<sup>2</sup>, and J.A. Wiles<sup>3</sup>.** <sup>1</sup>Corteva Agriscience<sup>TM</sup>, Bradenton, FL 34208, US. <sup>2</sup>Corteva Agriscience<sup>TM</sup>, Truderingerstr 15; 81677 München; Germany. <sup>3</sup>Corteva Agriscience<sup>TM</sup>, CPC2 Capital Park, Fulbourn, Cambridge, CB21 5XE, England.

Salibro<sup>TM</sup> (which contains Reklemel<sup>®</sup> active) is a new, highly effective and selective nematicide from Corteva Agriscience<sup>TM</sup> for the control of plant-parasitic nematodes. Specificity for nematodes coupled with absence of activity against the target sites of commercial nematicides suggests that Salibro<sup>®</sup> has a novel mode of action. It is the first member from the novel chemical class of sulfonamide nematicides. Salibro<sup>®</sup> has been extensively tested in laboratory, greenhouse, micro-plot and field trials in North America over the past years. In those trials Salibro<sup>®</sup> has proven extremely effective against a range of important plant-parasitic nematode species. This paper will focus on the fit of Salibro<sup>®</sup> in nematode management programs in root vegetable crops in North America. Over more than 5 years of field testing, Salibro<sup>®</sup> applied by various application methods according to the crop type, has demonstrated consistent and significant gall reduction on plant roots and benefits in marketable yield in carrot, potato and sweet potato crops. In carrots, pre-plant incorporated applications of Salibro<sup>®</sup> consistently reduced root galling by *Meloidogyne incognita* by 25-50% over the commercial standards which led to higher marketable yields. In potatoes, Salibro<sup>®</sup> applied in furrow at planting for Columbia root knot nematode (*Meloidogyne chitwoodi*) control followed by a Vydate <sup>®</sup> CL-V program increased marketable yields by 5 and 8% over the Vydate<sup>®</sup> CLV program alone and the competitive program, respectively. In sweet potatoes, Salibro<sup>®</sup> applied by pre-plant incorporation consistently controlled key root knot nematode species (*Meloidogyne incognita* and *Meloidogyne enterolobii*) and increased marketable yields by 10-20% versus the commercial standards tested. Overall, extensive field testing has demonstrated that Salibro<sup>®</sup> will offer an effective new tool for the management of root-knot nematode pests in several important root crops. <sup>®®®™</sup>Trademarks of Dow AgroSciences, DuPont, or Pioneer and their

EVALUATION OF DIFFERENT COVER CROPS AS A MANAGEMENT STRATEGY FOR *PRATYLENCHUS PENETRANS* AND *MELOIDOGYNE HAPLA* IN CARROT. **Thapa, Sita, K. Poley, and M. A. Quintanilla-Tornel.** Dep. of Entomology, Michigan State University, East Lansing, MI 48824.

Michigan ranks 4<sup>th</sup> nationally in carrot production with approximately 7,000 acres of carrots planted each year. Carrots in Michigan are valued at over \$21 million annually with a gross revenue of approximately \$6500 per acre. Plant parasitic nematodes can cause both quantitative and qualitative yield loss for carrot growers. Feeding by nematodes such as *Pratylenchus penetrans* (root lesion) and *Meloidogyne hapla* (Northern root knot) results in forked or stubbed carrots. A survey of carrot growers in Michigan in 2016 revealed that in a nematode infested field, 25% of

annual yields suffer from quality issues. Chemical nematicides are the primary source of control for nematodes in carrots, with a heavy reliance on a single pesticide: Vydate (Oxamyl; DuPont). As a non-chemical alternative, cover crops including oilseed radish (OSR) have been shown to reduce plant-parasitic nematode populations. Seed companies market OSR under brand names, however, only a few of these names are variety certified. Thus, it is not clear which radishes are truly genetically distinct (and may provide different benefits), and which are the same (and likely comparable in benefits). Because seeds are usually sold as variety-not-stated, the preservation of the underlying cultivar year-to-year is questionable. Michigan carrot growers need research-based information about the use of cultural strategies, like the use of cover crops, for nematode control. The main objectives of this project are 1) To evaluate the host status of 12 different cover crops [Biofum summer mustard, Black oats, Cappuchino mustard, OSR (Defender, Ecotill, Respect, and Scavenger) Jupiter wheat, Oats, Pearl millet, Sorghum Sudan grass, and Sunn hemp] in the growth chamber; 2) To identify the genetic differences within the OSR cultivars through DNA sequencing; and 3) To evaluate the efficacy of the above-mentioned cover crops in *P. penetrans* and *M. hapla* management in a carrot field established in Hart, MI. A Preliminary growth chamber trial determined that Sunn hemp, Sorghum, and Jupiter wheat are good hosts for *P. penetrans*, and Biofum summer mustard, OSR (Defender, Ecotill, and Respect) are poor hosts for *P. penetrans*. Similarly, we found that Cappuchino mustard, Biofum summer mustard, OSR (Defender, and Scavenger), and Sunn hemp are poor hosts for *M. hapla*. This project is in the starting phase, more growth chamber trials and field trials in the near future will provide useful information on above-mentioned cover crops as a management strategy for *P. penetrans* and *M. hapla*.

## CAN WINTER COVER CROPS INCREASE NEMATODES SUPPRESSION? Timper, Patricia<sup>1</sup>, W.C. Johnson<sup>1</sup>, T. Strickland<sup>1</sup>, and G. Jagdale<sup>2</sup>. <sup>1</sup>USDA ARS, P.O. Box 748, Tifton, GA 31793. <sup>2</sup>Plant Pathology Dept., University of Georgia, Athens, GA 31793.

Previous research has shown that high levels of soil carbon from plant residue enhances general suppression of plant- parasitic nematodes. We hypothesized that planting winter cover crops would increase suppression of Meloidogyne incognita in the spring compared to a weedy fallow. The experiment was conducted over three years (2016-2018) and two field sites (Belflower Farm and Jones Farm); the soil at both sites was a loamy sand. There were three cover crop treatments at each farm: a weedy fallow, rye, and a rye/crimson clover mix (Belflower) or hairy vetch (Jones). The design was a randomized block with four replicates for each treatment. The cover crops were rolled in the spring to prevent further growth and the residue left on the soil surface. Just before planting cotton, soil was collected from each plot to identify nematode functional groups and to determine suppression of *M. incognita* using two different bioassays. One bioassay compared survival of second-stage juveniles (J2) and the other compared nematode reproduction on cotton among the cover crop treatments. For the survival bioassay, half the soil from each plot was heated for 1 hr at 65 C to reduce fauna and flora, and survival of J2 was compared in natural vs heated soil. The number of predatory and omnivorous nematodes did not differ among the cover crops except at the Jones Farm in 2016 when more omnivores were present in the hairy vetch than in the weedy fallow treatment. Survival of M. incognita J2 was lower in natural compared to heated soil in all years at the Jones Farm and in 2016 at the Belflower Farm indicating that the soil community was suppressive; however, survival did not vary among cover crop treatments.Reproduction of M. incognita was suppressed by 54-66% in soil planted to both rye and rye/clover compared to the weedy fallow at the Belflower Farm; there was no effect of cover crop on nematode reproduction at the Jones Farm. This research showed that winter cover crops can enhance general suppression of plant-parasitic nematodes; however, the suppression varies among field sites.

### REPRODUCTION ABILITY AND GROWTH EFFECT OF PIN NEMATODE, PARATYLENCHUS NANUS, ON SELECTED CULTI-

VARS OF FIELD PEA. Upadhaya, Arjun and G. P. Yan. North Dakota State University, Department of Plant Pathology, Fargo, ND 58108. Pin nematodes, Paratylenchus spp., are among the smallest of the plant-parasitic nematodes and are associated with diverse plant species. Paratylenchus spp. can parasitize plant roots and cause detrimental effects on plant growth. Soil surveys across pea fields in North Dakota identified Paratylenchus spp. as the dominant plant-parasitic nematodes based on the frequency of occurrence and population densities. Populations of Paratylenchus spp. from three farms wereidentified as P. nanus only based on morphometric measurements and molecular examinations. However, the effect of *P. nanus* populations on field pea plant growth and yield is not known. Hence, greenhouse experiments were conducted to determine the reproduction ability and growth effect of P. nanus from North Dakota on field pea cultivars. Reproduction of P. nanus was determined on seven field pea cultivars using naturally infested field soils at low (1,500/kg of soil) and high (4,500/kg of soil) initial population densities of pin nematode. Nematode effect on plant growth and seed yield was evaluated at 4,500 P. nanus/kg of soil by artificially inoculating P. nanus on six field pea cultivars. Average reproductive factor of P. nanus was found to be greater at the low density than the high density of the nematode across all the cultivars. At the low population density, reproductive factor values ranged from 1.10 to 11.20, while at the high density, they ranged from 1.20 to 2.50. In experiments with evaluation of *P. nanus* effects on cultivar growth, the nematode (4,500 P. nanus/kg of soil) caused reduction (P < 0.05) of plant height in most cultivars tested, and also significantly reduced dry shoot weight and dry seed weight in some experiments. Plant height and shoot weight reductions were the highest in the cultivar Arcadia, up to 37% and 53%, respectively, with a dry seed weight reduction up to 32%. This study demonstrated the negative impact of P. nanus on field pea plant growth and seed weight in controlled greenhouse conditions, which is an important step towards developing effective management strategies to improve the productivity of this leguminous crop.

A NOVEL SPECIES OF RNA VIRUS IDENTIFIED IN THE MIGRATORY ROOT LESION NEMATODE *PRATYLENCHUS PENE-TRANS*. Vieira, Paulo<sup>1,2</sup> and L. G. Nemchinov<sup>1</sup>. <sup>1</sup>Molecular Plant Pathology Laboratory, Agricultural Research Service, United States Department of Agriculture, Beltsville, Maryland. <sup>2</sup>School of Plant and Environmental Sciences, Virginia Tech, Blacksburg, Virginia.

In recent years, several new viruses infecting plant-parasitic nematodes have been described. All of them were found in sedentary nematode species. The root lesion nematode *Pratylenchus penetrans* is a migratory endoparasitic species that attacks a broad range of plants and has an economic impact in crops worldwide. In this work, while analyzing our transcriptomic datasets of *P. penetrans*, we have identified genome sequences of an unknown positive-sense single- stranded RNA virus. The complete viral genome was obtained by *de novo* assembly and by sequencing of the 5'/3'RACE- amplified cDNA ends. The virus was provisionally named root lesion nematode virus 1 (RLNV1). The 8,614-nucleotide long genome sequence encodes a single large polyprotein with conserved domains characteristic for the families *Picornaviridae*, *Iflaviridae* and *Secoviridae* of the order *Picornavirales*. BLAST, domain search and phylogenetic analyses demonstrated that RLNV1 is a novel species, related to the recently identified sugar beet cyst nematode virus 1 and potato cyst nematode picorna-like virus. *In situ* hybridization with a DIG-labeled DNA probe confirmed the presence of the virus within the nematodes. Negative strand-specific RT-PCR assay detected RLNV1 RNA in nematode total RNA samples, confirming that viral replication occurs in *P. penetrans*. To our knowledge, RLNV1 is the first virus identified in migratory nematode species of genus *Pratylenchus*. The effect of the virus infection on the viability of the nematodes and their development has yet to be clarified.

TRANSCRIPTOME ANALYSIS OF A *RADOPHOLUS SIMILIS* POPULATION ISOLATED IN HAWAII. **Vieira, Paulo<sup>1</sup>, R. Myers<sup>2</sup>, C. Mello<sup>2</sup>, T. Matsumoto<sup>2</sup> and K. Kamo<sup>3</sup>**. <sup>1</sup>School of Plant and Environmental Sciences, Virginia Tech, Blacksburg, Virginia. <sup>2</sup>Daniel K. Inouye U.S. Pacific Basin Agricultural Research Center, USDA ARS, Hilo, Hawaii. <sup>3</sup> Floral and Nursery Plants Research Unit, U.S. National Arboretum, USDA ARS, Beltsville, Maryland.

The burrowing nematode, *Radopholus similis*, is an economically important pathogen that inflicts damage and yield loss to a wide range of crops. This migratory endoparasite is widely distributed in Hawaii and causes extensive destruction to the root systems of banana and anthurium. In banana, burrowing nematode infestations can lead to decline and toppling of large plants. Infected anthuriums become severely stunted resulting in smaller flowers and yield reductions of up to 50%. To obtain insight into the transcriptome of this species, we used Illumina mRNA sequencing analysis of a mixed population originally collected from a commercial anthurium farm in Hilo, Hawaii. Over 106 million paired end reads were obtained, and a *de novo* transcriptome assembly resulted in a total of 44,478 transcripts. Homology searches showed significant hit matches to 57.6% of the total number of transcripts using different public databases. Using different comparison analyses, several candidate effector genes were identified, both pioneer genes and transcripts with similarities to other nematode parasitism genes. A small set of candidate effector genes were localized within the esophageal glands of the nematode by *in situ* hybridization. These analyses provide additional transcriptome data for a migratory and economically important plant-parasitic nematode.

### WILL ENHANCEMENT OF THE BIOFUMIGATION EFFECT COMPROMISE THE SOIL HEALTH BENEFITS OF BRASSICA COV-ER CROPS? **Philip Waisen, K.-H. Wang and B.S. Sipes**. University of Hawaii at Manoa, Honolulu, HI 96822.

Brassica cover crops like oil radish (Raphanus sativus, OR) and brown mustard (Brassica juncea, MS) produce biofumigants toxic to plant-parasitic nematodes (PPNs) but at the same time are popular for soil health management. Objectives of this project were to 1) compare biofumigation effects of OR and MS on soil health, and 2) examine relationships between biofumigation and soil health indicators. Two field trials (Trials I and II) were conducted in which the biofumigant crops were subjected to various termination methods: 1) no-till (NT); 2) no-till followed by tissue maceration (MNT); 3) MNT followed by covering with black plastic (NTBP); 4) till only (T); 5) maceration followed by tillage (MT); and 6) MT followed by covering with black plastic (MTBP). Termination methods were compared to a bare ground control (BG). Soil samples were collected 1 week after biofumigant crop termination and at monthly intervals during a zucchini (Cucurbita pepo) crop. Extracted nematodes were subjected to nematode community analysis as soil health indicators. Performing biofumigation using both OR and MS had no negative impact on free-living nematodes. In fact, OR enhanced nutrient enrichment throughout a zucchini crop and MS enhanced nutrient enrichment for up to 1 month after biofumigation in Trial II. Glucose levels in the soil were analyzed as a proxy indicator of myrosinase (Myr) activity during biofumigation. Sulfate, a byproduct of biofumigation, was also analyzed as a biofumigation indicator. Canonical correspondence analyses (CCA) were performed to detect relationships between species variables (richness, abundance of nematode trophic groups) and environmental variables (nematode community indices, Myr activity and soil sulfate concentration). In Trial I, a strong positive relationship between Myr activity and abundance of bacterivorous, fungivorous, omnivorous and predatory nematodes was detected (First two canonical analysis explained 95.7% of variance). In the same CCA, Myr activity was negatively related to abundance of total PPNs. In Trial I, when biological activity was not stopped immediately at sampling, Myr activity was not negatively related to abundance of PPNs, but sulfate concentration, which is more stable in the soil, was negatively related to abundance of root-knot nematodes and positively related to the abundance of bacterivorous and predatory nematodes, and nematode enrichment index (First two canonical analysis explained 89.0% of variance). In summary, biofumigation is very effective in suppressing PPNs while enhancing free-living nematodes that are important in enhancing soil nutrient enrichment.

### NEMATODES ASSOCIATED WITH SUGARCANE GROWN IN FLORIDA SPODOSOLS. **Waldo, Benjamin<sup>1</sup>, G. S. Swanson<sup>2</sup>, A. W. Habteweld<sup>1</sup>, M. L. Mendes<sup>1</sup> and W. T. Crow<sup>1</sup>.** <sup>1</sup>Entomology and Nematology Dept., P.O. Box 110620, Building 970, Natural Area Dr. University of Florida, Gainesville, FL 32611-0210. <sup>2</sup>University of Florida Hendry County Extension, 1085 Pratt Blvd. P.O. Box 68 LaBelle, FL 33975-0068.

Sugarcane (*Saccharum* sp.) is one of the most important crops in Florida in terms of acreage and value. Most sugarcane production in Florida occurs south of Lake Okeechobee in the Everglades Agriculture Area on fertile Histosol soil. As land in the everglades is restored to natural ecosystem conditions, less Histosol acreage is available for cultivation of crops like sugarcane. Florida sugarcane production is shifting to sandy Spodosols, which are a more suitable environment for many plant-parasitic nematodes. Little work has been devoted to impacts of nematodes on sugarcane systems in Florida. In order to begin better understanding the relationship between nematodes and sugarcane grown on Spodosols in Florida, plant-parasitic nematodes were identified from composite soil samples from four Spodosol sugarcane farms in south Florida. Fifteen plant-parasitic genera were found associated with sugarcane on these farms. Of the genera encountered, *Meloidogyne, Pratylenchus, Trichodorus*, and *Hemicycliophora* were most common and could be important pathogens of sugarcane. Host differential testing indicated *Meloidogyne incognita* race 2 was present in one field of our survey. Further testing of the pathogenicity of these nematodes on sugarcane is required to improve understanding of risks of nematodes to Florida sugarcane production.

SOYBEAN CYST NEMATODE ATTRACTION TO PLANTS AND CHEMICALS. **Wang, Congli, J. You, C. Hua, Y. Hu, C. Li, Y. Jiang, M. Huang, F. Pan.** Key Laboratory of Mollisols Agroecology, Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences, Harbin 150081, China.

The soybean cyst nematode (SCN), Heterodera glycine, is one of the most economically important soybean pathogen. H. glycine is an obligate endoparasitic nematodes. The infective second stage juvenile in the soil is capable of detecting signal gradient to find a host, to locate to root tips and then to penetrate the root. However, little is known about how SCN perceive chemical gradients from root or root micro-environment and about nematode genes that are involved in the early stages of parasitism. Based on our previous and current studies by using Pluronic F-127 gel system, we investigated nematode attraction to different plants and potential chemical cues (acid, alkaline, salt and amino acid). We found that SCN are attractive only to soybean but not tomato, rice and other plant roots which might explain narrow host range for SCN. Chemotaxis studies showed SCN attraction to acid (HAc), alkaline (NaOH), salts and different types of amino acids with dosage effect. The optimal acidic pH range for SCN attraction is 4.98-5.46. Two optimal alkaline pH ranges were  $8.40 \pm 0.01$ - $8.78 \pm 0.02$  and  $9.52 \pm 0.05$ - $9.99 \pm 0.03$ , respectively. The optimum concentrations of Na+ and Cl+ were  $85.24 \sim 221.52$  mM and 170.89~255.87 mM, respectively. Through differential expression of transcriptome analysis (RNA-seq), nematode genes involved in attractiveness of plants, acid, alkali and salts were identified, e.g. genes associated with nucleoside diphosphate kinase, metalloprotease (Ftsh), thiazole synthase, superoxide dismutase, cation transporting ATPase, annexin and phospholipid-transporting ATPase, ligand-gated ion channel and CBN-RSKN-2 protein which have been verified by RT-qPCR. Further functional verification of these genes by RNA interference are ongoing. All these studies will be utilized to establish one model system of early interaction between plant and nematode. Information on the chemical cues, signals and genes will gain more insight about how the host is recognized and using this understanding for novel approaches to nematode control.

## THE RELATIONSHIP BETWEEN SOIL-BORNE DISEASE PRESSURE AND SOIL HEALTH INDICATORS AS AFFECTED BY BIO-FUMIGATION. Koon-Hui Wang, P. Waisen, and J. Silva. University of Hawaii at Manoa, Honolulu, HI 96822.

Literature on biofumigation suggested that increasing soil moisture might increase the biofumigation effect against soil-borne pests and pathogens. Two forms of biofumigation using crop residues of brown mustard, Brassica juncea, incorporated into the soil and covered with solarization mulch (clear plastic) for one week were examined for potential suppressive effects against soil-borne fungi such as Rhizoctonia solani and Fusarium oxysporum f. sp. lettuca on Manoa lettuce (Lattuca sativa). The specific objective was to examine relationships between soil-borne disease suppression, soil health conditions, and biofumigation. Three preplant treatments compared were: 1) biofumigation by amending 1.5 mt dry matter/ha of brown mustard biomass (M) into soil without soil moisture adjustment (soil moisture = 20.8%), 2) biofumigation by amending soil with M followed by adding water to adjust soil moisture to 40.8 % (MW), and 3) a no biofumigation control (C). Treatments were arranged in randomized complete block design with 4 replications. Manoa lettuce was seeded 2 weeks after biofumigation. Since sulfate is a byproduct of the hydrolysis of glucosinolate during the biofumigation process, increase in soil sulfate concentration after biofumigation compared to before was used as an indicator for the efficacy of biofumigation. Only M increased sulfate concentration in the soil but not MW or C. Adjusting soil moisture to 40.8% (MW) might have disrupted the biofumigation effect. Nonethe-less, both M and MW increased microbial activities based on the Solvita respiration test compared to C. Although Manoa lettuce had larger canopy width in the MW than M and C, its marketable yield was not different among treatments. In fact, MW had higher unmarketable weight (mostly associated with Fusarium wilt, bottom rot and Ca deficiency) than M. Lack of significant difference in the incidence of Fusarium wilt on Manoa lettuce was possibly due to low disease pressure. The first two Canonical variables of a multivariate analysis among disease incidence, lettuce yield, and abundance of plant-parasitic nematodes with environmental variables (soil sulfate concentration, soil microbial respiration, enrichment index (EI), channel index (CI), percentage of bacterivorous and fungivorous nematodes in the nematode communities) explained 96.7% of the variation. Percent of Manoa lettuce showing Fusarium wilt was positively related to CI, but negatively related to % bacterivorous nematodes and changes in sulfate concentration. No relationship was observed between % Fusarium wilt and EI or soil microbial respiration. In summary, adjusting soil moisture to 40.8% for biofumigation using brown mustard, though improved the green manure effect of the cover crop residues, interfered with its biofumigation effects against Fusarium wilt on Manoa lettuce. This study also showed that suppression of soil-born fungal pathogens through biofumigation cannot be depicted by soil microbial respiration in general, rather it relies on efficacy of the hydrolysis during biofumigation as well as enhancement of bacteria activities in the soil.

### EVALUATING COMBINATIONS OF PRODUCTS FOR MANAGEMENT OF ROOT-KNOT NEMATODE ON ANNUAL CROPS. **Westerdahl, Becky B<sup>1</sup>**. <sup>1</sup>University of California, Davis, CA 95616.

Twofieldtrialsevaluatednewproductsformanagementofroot-knotnematode, *Meloidogyneincognita*, oncarrots. Trialsincludedan untreated control and were conducted in a randomized complete block design with 5 replicates per treatment. Twenty-three treatments in the first trial included: fluopyram (Velum, Bayer); TECA Fulvic, TECA Nemat- X, and TECA Root from IG Agrosolutions; fluensulfone (Nimitz, Adama); fluazaindolizine (Salibro, DuPont); metam potassium (K-pam HL, AMVAC); QL Agri (*Quillaja*, Desert King); FBS (soil organic acids, Fbsciences); Cedroz (geraniol and thymol, Eastman Chemical Company); and CrossLink (tergatol, amino acids, Beem Biologics). Seventeen treatments in the second trial included: fluensulfone, fluopyram, *Paecilomyces lilacinus* (BioAct, Bayer); *Paecilomyces lilacinus* (MeloCon, Certis); fluensulfone; ISO (*Guayule* extract), SA Propel (soil organic acids), and Focus (bacterial complex) from Beem Biologics; Groactive (*Yucca* and *Quillaja*, Desert King); and 1,3-dichloropropene (Telone II, Dow AgroSciences). Products were tested either alone, or in combination with other products. All products evaluated increased yields compared to the untreated control. Compared to individual products alone, yields increased with combinations of Velum plus TECA products; of QL Agri plus FBS; of K-pam plus Cedroz; of Velum with BioAct, and of ISO with SA Propel.

PATHOGENICITY OF *APHELENCHOIDES BESSEYI* ON CHRYSANTHEMUM. Wheeler, Lindsay and W.T. Crow. Entomology and Nematology Department, University of Florida, Gainesville, FL 32611.

Nematodes in the genus *Aphelenchoides* are known to primarily feed on fungi, but there are a few species that also feed on the foliar tissue of plants. Of these plant-parasitic species, *A. ritzemabosi* is the one most closely associated with pathogenicity on chrysanthemum (*Chrysanthemum indicum*), and nearly all literature regarding foliar nematode parasitism of chrysanthemum centers on this species. However, a recent outbreak of *A. besseyi* on chrysanthemum in a Florida nursery has prompted interest in a new host-nematode association. In 2018 and 2019, multiple experiments were conducted to gather data on *A. besseyi*'s pathogenicity on 'Olympia' chrysanthemum grown from cuttings. These experiments compared biomass and quality of plants inoculated with *A. besseyi* to non-inoculated plants and recorded associated symptoms. At the conclusion of each experiment, the plants were harvested at the soil line and weighed to record the top mass. The leaves of each plant were then removed and weighed to record the leaf mass. In two of the experiments, the plants were also given rankings based on their quality of appearance. In every experiment, the inoculated plants consistently displayed reduced weight, decreased vigor, and a lower quality of appearance compared to non- inoculated plants. These findings supported the hypothesis that *A. besseyi* is a pathogen of chrysanthemum.

NEMATOLOGY COMPETENCIES AND MULTI-DISCIPLINARY INTERACTIONS IN DEVELOPING A NOVEL NEMATICIDE (REKLEMEL<sup>™</sup> ACTIVE) ON A GLOBAL SCALE. **Wiles, John A.<sup>1</sup>, T. Thoden<sup>2</sup>& J. Temple<sup>3</sup>**. <sup>1</sup>Corteva Agriscience<sup>TM</sup>, CPC2 Capital Park, Fulbourn, Cambridge, CB21 5XE, England. <sup>2</sup>Corteva Agriscience<sup>TM</sup>, Truderingerstr 15; 81677 München; Germany. <sup>3</sup>Corteva Agriscience<sup>™</sup>, Bradenton, FL 34208, US.

The management of plant-parasitic nematodes (PPNs) is a key issue globally, and they are well known yield robbers of many important crops all around the world, resulting in large economic losses (estimated >\$100 billion) reducing food production, both in quantity and quality. The scientific literature demonstrates a large research effort into finding new solutions but few eventually become available to growers. There are many reasons for this, from potency, selectivity, environmental behavior, scalability, registerability, to economic perspectives. In this paper we will describe learnings from developing a novel nematicide (Reklemel<sup>™</sup> active) on a global scale from discovery to field uses. We will highlight how various nematology competencies and techniques, from molecular, physiological to whole-organism assays, are needed to investigate how a product acts on PPNs themselves, especially in terms of mode-of-action, life-stages and species sensitivity ranges, and selectivity to other rhizosphere inhabiting organisms. We will also examine how interdisciplinary skills in applied field nematology, soil science, crop agronomy and application technology are critical aspects to understand, both mechanistically and practically, how a product behaves in the soil environment and can be used in a way that achieves effective concentrations against PPNs in the crop rhizosphere. Our experiences highlight the diversity of competencies needed to bring new nematode management solutions to market and how research networks are to be encouraged to reach this aim.

ENTOMOPATHOGENIC NEMATODE LEARNING. **Willett, Denis S.<sup>1</sup>, L.W. Duncan<sup>2</sup>, H.T. Alborn<sup>3</sup>, L.L. Stelinski<sup>2</sup>, C.C. Filgueiras<sup>1</sup>.** <sup>1</sup>Department of Entomology, Cornell AgriTech, Cornell University. <sup>2</sup>Department of Entomology and Nematology, CREC, University of Florida. <sup>3</sup>USDA ARS CMAVE.

Entomopathogenic nematode infection of host insects is a group effort; a cohort of infective juveniles is needed to overcome the host's immune system. To be able to coordinate host attack, entomopathogenic nematodes have developed the ability to respond rapidly to volatile cues in their environment - especially if they have had prior experience with those cues. Here we discuss behavioral plasticity of entomopathogenic nematodes in the context of their belowground environment with an emphasis on the ramifications of such learning for other organisms. Specifically, we show how entomopathogenic nematode learning multiplies the efficacy of plant defenses belowground and discuss opportunities for appropriating this ability for applied pest control in agriculture.

# ASSESSING WHETHER COVER CROPS AFFECT HATCHING AND CHEMOTAXIS OF THE SOYBEAN CYST NEMATODE. Wlezien, Elizabeth, C. J. Harbach, and G. L. Tylka. Department of Plant Pathology and Microbiology, Iowa State University, Ames, IA 50011.

A possible new management option for soybean cyst nematode (SCN), *Heterodera glycines*, is the use of cover crops. Certain crops have been reported to serve as trap crops for other cyst nematodes, attracting juveniles that penetrate the roots but cannot feed and subsequently starve. To investigate the possibility of this phenomenon with SCN, experiments were conducted to study effects of cover crops on hatching and chemotaxis of SCN juveniles. The premise for these experiments is the roots of trap crops may stimulate hatching and/or attract hatched juveniles of the nematode. Treatments used in the experiments were several different broadleaf cover crops, cereal rye (*Secale cereal*), and annual ryegrass (*Lolium multiflorum*). An SCN-susceptible soybean variety, nonhost tomato, and unplanted soil were included as three control treatments. Root exudates and soil leachates (RE/SL) were collected from the treatments after growing for 30 days in the greenhouse in a noninfested soil mix. The RE/SL were filter sterilized then used in hatching and chemotaxis experiments. To assess effects on hatching, eggs were incubated in RE/SL at 25°C in 6-well tissue culture plates for 14 days, and hatched juveniles were counted at 3, 7, and 14 days. Unhatched eggs remaining at 14 days were counted and percent hatching was calculated. Deionized water and 5mM zinc sulfate were neutral and positive control treatments, respectively. Hatching was 37-42% in RE/SL from crimson clover, *Trifolium incarnatum*, which was significantly greater than hatching in RE/SL from the unplanted control (17%-23%). The RE/SL form all other broadleaf cover crop treatments and the cereal rye and annual ryegrass treatments did not affect hatching. To study the effects of RE/SL on chemotaxis of the SCN juveniles, RE/SL were decanted into a randomly selected reservoir in a lane of a chemotaxis microfluidic chip and deionized water was placed in the reservoir on the opposite end of the same lane.

Freshly hatched SCN juveniles were placed in the nematode entry port in the center of the lane and the chips were incubated on a level surface at 25°C for 24 hours, then the number of juveniles that moved towards or away from the RE/SL were counted. The two control treatments in the experiments were 0.5M potassium nitrate and 0.5M calcium chloride, which attracted and repelled the SCN juveniles,

respectively. About 20% of the juveniles were attracted toward the RE/SL from Aroostook cereal rye and from Rootmax annual ryegrass and 45% were attracted to the potassium nitrate control. There was no attraction to any of the other cover crop RE/SL treatments, and none of the RE/SL from cover crops repelled the juveniles. The hatch stimulation of RE/SL from crimson clover and the attraction of juveniles to the RE/SL of the cultivars of cereal rye and annual ryegrass may indicate that these plants have potential to serve as trap crops for SCN.

### CURRENT RESEARCH STATUS ON MOLECULAR CHARACTERIZATION AND DIAGNOSTICS OF STUBBY ROOT NEMATODE SPECIES IN THE UNITED STATES. **Yan, Guiping.** North Dakota State University, Department of Plant Pathology, Fargo, ND 58108.

The ectoparasitic stubby root nematodes (Paratrichodorus and Trichodorus) are important plant-parasitic nematodes that affect many crops. They can be particularly devastating to potato due to their ability to transmit Tobacco rattle virus causing corky ringspot disease in potato. During 2015 to 2018, a total of 251 field soil samples and 17 nematode suspensions collected from ten states in the United States were examined for the presence of stubby root nematodes (SRN). They were detected in 53% of the nematode suspensions and in 51% of the soil samples with population densities of 10-320 nematodes/200 g of soil. Ribosomal DNA sequencing revealed the presence of four species, namely, Paratrichodorus allius, P. minor, P. porosus and Trichodorus obtusus. The species P. allius was detected in soil samples from six of the states, demonstrating that it is a prevalent virus vector in the major potato production areas. DNA sequences were characterized by analyzing D2-D3 of 28S rDNA, 18S rDNA, and ITS rDNA regions. Intra- and inter-species variabilities were higher in ITS than 18S and D2-D3. These four species formed a monophyletic group, with P. allius more closely related to P. porosus than P. minor and T. obtusus based on phylogenetic analysis. Indel variation of ITS2 rDNA was observed in P. allius populations from the same geographic regions. Morphological identification of SRN species is laborious and requires an extensive training in nematology. For efficient diagnosis, a conventional species-specific PCR assay targeting ITS1 rDNA was developed for detection and identification of P. allius from single nematodes and soil nematode communities. A multiplex PCR assay using one universal forward primer and four species-specific reverse primers was developed to simultaneously identify the four SRN species in one PCR reaction. A diagnostic method for direct detection and quantification of P. allius from field soil DNA using TaqMan probe and SYBR Green real-time PCR assays was developed to assist the potato industry in management of this important virus vector. Key factors associated with SYBR Green real-time PCR quantification of P. allius using soil DNA were evaluated, including nematode body sizes, soil pre-treatments, additional soil DNA purification steps, use of a PCR enhancer, number of DNA extractions for a soil sample, and types of standard curves used in the quantification assay. These factors are important to consider during the setup or optimization of real-time PCR based quantification of plant- parasitic nematodes from soil DNA. The genetic diversity will provide more information for understanding the evolutionary relationships of SRN species and the molecular assays will provide rapid identification and quantification of these species in infested fields for effective management of these nematode pests.

CHARACTERIZATION OF AN EMERGING ROOT-KNOT NEMATODE *MELOIDOGYNE ENTEROLOBII* IN NORTH CAROLINA, USA. **Ye, Weimin<sup>1</sup>, S. R. Koenning<sup>2</sup>, Y. Zeng<sup>3</sup>, K. Zhuo<sup>4</sup> and J. Liao<sup>4</sup>**. <sup>1</sup>Nematode Assay Section, Agronomic Division, North Carolina Department of Agriculture & Consumer Services, Raleigh, NC 27607. <sup>2</sup>Department of Plant Pathology, North Carolina State University, Raleigh, NC 27695. <sup>3</sup>Department of Plant Protection, Zhongkai University of Agriculture and Engineering, Guangzhou, 510225, China, <sup>4</sup>Department of Plant Pathology, South China Agricultural University, Guangzhou, 510642, China.

Meloidogyne enterolobii Yang & Eisenback, 1983 was originally described from the pacara earpod tree (Enterolobium contortisiliquum (Vell.) Morong) in China and later was found in over 30 countries, mainly in tropical areas. It is considered to be the most damaging species of root-knot nematodes (RKNs) in the world because of its wide host range, aggressiveness, and ability to overcome the resistance that has been developed against RKNs in many crops. In the USA, it was first detected from ornamental plants in Florida in 2001, but is now confirmed in North Carolina, South Carolina and Louisiana, probably through movement of infested plants. From 2006 to 2019, thousands of RKN populations were collected from North Carolina field crops, ornamental plants and turfgrasses for species identification in the Nematode Assay Laboratory in the North Carolina Department of Agriculture & Consumer Services. Root systems showing galling symptoms were dissected under the microscope and females were obtained for DNA analysis. When samples were submitted as soil only, the second-stage juveniles or males were used instead. Molecular characterization was performed by PCR using species-specific primers and DNA sequencing on the ribosomal DNA 18S, ITS and 28S D2/D3, intergeneric spacer, RNA polymerase II large subunit, and mitochondrial DNA cytochrome oxidase gene subunit II. About 100 representative RKN populations from North Carolina were characterized and identified as M. enterolobii. Eight samples from China where the species was originally described were included in this study for identity confirmation. Root-knot nematode bioassay using differential hosts including tomato, peanut, cotton, watermelon, pepper, and tobacco were conducted on two populations of M. enterolobii from soybean and cotton, respectively. Reproduction occurred on all hosts except for peanut, which is consistent with reports for M. enterolobii and M. incognita race 4. As of June 2019, M. enterolobii was confirmed from limited fields in Columbus, Greene, Harnett, Johnston, Lenoir, Nash, Pitt, Sampson, Wayne and Wilson counties in North Carolina. Meloidogyne enterolobii has become an emerging RKN species and has caused severe damage to crops, especially sweet potato, in North Carolina.

# *XIPHINEMA AMERICIUM*-SPECIES COMPLEX DIVERSITY IN THE PACIFIC NORTHWEST. **Zasada, Inga<sup>1</sup>, A.B. Peetz<sup>1</sup>, M.L.C. Kitner<sup>1</sup>, T.A. Forge<sup>2</sup>, and C.N. Hesse<sup>1</sup>. <sup>1</sup>USDA ARS, 3420 NW Orchard Ave., Corvallis, OR 97330. <sup>2</sup>Agriculture-Agri Food Canada, Summerland, British Columbia.**

Dagger nematodes (*Xiphinema americanum*-species complex) are commonly found in perennial crops including grapes, blueberries, cherry, apple, and raspberry in the Pacific Northwest (PNW) of the United States and Canada. The risk of these nematodes to fruit production in the PNW is that they are vectors of several NEPO viruses, some of which are rarely found (Tomato and Tobacco Ringspot Viruses) and others that are more widely prevalent (Cherry Ringspot virus). Viruses can be introduced into fields, vineyards, and orchards via contaminated planting material. Spread within a location is mediated by dagger nematodes, leading to the question of whether all *X. americanum*-species complex nematodes are capable of vectoring viruses. This research was undertaken to begin to characterize the diversity of dagger nematodes present in the PNW, with the ultimate goal of understanding the virus-vectoring potential of this group of nematodes. Populations of *X. americanum*-species complex were collected from 10 fruit production fields in the region. The populations were then morphologically and molecularly characterized. For morphological characterization, 10 individuals from each population were measured and then a character phylogeny was constructed. The character-based 83 phylogeny indicated substantial overlap of morphometric characters among populations. For molecular characterization, DNA from the same 10 individuals used for morphology was extracted. Genus-specific primers targeting a 2,800 base pair (bp) mtDNA region were designed from accessions deposited in GenBank which included the entire mt genome, portions of the cytochrome oxidase 1 (CO1) gene, partial sequences from the 3' end of the ribosomal small subunit (mtSSU), and the cytochrome b (CYTB) gene. PCR amplicons from individual isolates were cloned and sequenced in multiplereactions. Preliminary results indicate that there are at least five species from this complex present in the PNW. The virus vectoring capacity of these species remains unknown.

INSIGHT FROM THE WILD — DISSECTION OF BROAD-SPECTRUM RESISTANCE TO SOYBEAN CYST NEMATODE (SCN) US-ING AN INTEGRATIVE APPROACH. **Zhang, Hengyou<sup>1</sup>, N. Mittal<sup>1</sup>, X. Li<sup>2,3</sup>, Z. Li<sup>4</sup>, L. Li<sup>5</sup>, E. Davis<sup>5</sup>, C. Li<sup>6</sup>, and B-H. Song<sup>1</sup>. <sup>1</sup>Department of Biological Sciences, University of North Carolina at Charlotte, Charlotte, NC 28223, USA. <sup>2</sup>Plant for Human Health Institute, North Carolina State University, Kannapolis, NC 28081, USA. <sup>3</sup>Department of Plant and Microbial Biology, North Carolina State University, Raleigh, NC 27695, USA. <sup>4</sup>Institute of Plant Breeding, Genetics and Genomics and Department of Crop and Soil Sciences, University of Georgia, GA 30602, USA. <sup>5</sup>Department of Plant Pathology, North Carolina State University, Raleigh, NC 27607, USA. <sup>6</sup>Syngenta Research Triangle Park, Durham, NC 27709, USA.** 

Soybean cyst nematode (*Heterodera glycine*, SCN) is the most damaging parasite that causes high losses in soybean production worldwide. Rapid shifts in SCN races and lack of new resistant resource composition represent two of the biggest challenges for SCN management. To meet these challenges, we identified a novel *Glycine soja* genotype, S54, showing broad-spectrum resistance to two SCN races (2 and 5) and elucidated the underlying resistance mechanism by comparing transcriptome and metabolome responses in resistant and susceptible genotypes. This global analysis identified a core set of differentially expressed genes and metabolites, including  $Ca^{2+}$  and salicylic acid (SA)-related signalling genes and phenolic compounds, that commonly responded to the two races. Candidate enzyme-encoding genes involved in phenolic biosynthesis were identified. This study showed that positive regulation of  $Ca^{2+}$ -SA signalling pathways and enhanced phenolic biosynthesis contributed to the enhanced resistance of S54, which shed light on SCN resistance and provided a new perspective on plant chemical defence.

ENGINEERING BACTERIA AS DELIVERY VEHICLES OF THE DEFENSE-INDUCING PEPTIDE STPEP1 IMPROVES ROOT-KNOT NEMATODE RESISTANCE IN POTATO **Zhang, Lei and Gleason, C.A.**Department of Plant Pathology, Washington State University, Pullman, WA 99164, USA.

Root-knot nematodes with their ability to infect most crop plants and worldwide distributions have caused billions of dollars losses in agriculture. The Columbia root-knot nematode (CRKN, *Meloidogyne chitwoodi*) is a devastating potato pest in the Pacific Northwest (PNW) of the United States. This nematode infects both potato roots and tubers, causing significant losses in potato yields and marketability. Due to lack of resistant potato cultivars, growers heavily reply on chemical nematicides to control this nematode. This research is aimed at developing alternative approaches for root-knot nematode management. We have focused on the plant elicitor peptides (Peps), which are small plant signal peptides and induce plant defense response against broad spectrum of pathogens. Our studies found out that application of the chemically synthesized potato StPep1 on potato plants significantly reduced the CRKN infections by 50% in terms of number of galls. In order to develop an economical approach to deliver the StPep1 to plant roots, we genetically engineered the beneficial soil-borne bacterium *Bacillus subtilis* to synthesize and secrete the StPep1. Our data showed that pre-treatment of potato plants with the StPep1-secreting *Bacillus* significantly reduces root-knot nematode infections. Furthermore, we performed RNA-seq analysis on StPep1-treated potato roots and discovered groups of root-specific defense marker genes. In summary, our studies demonstrate that the engineered *Bacillus* secreting StPep1 triggers plant immunity and leads to enhance potato resistance to root-knot nematodes.

### BORON-BASED COMPOUNDS AS NOVEL DUAL ACTION MOLECULES FOR THE CONTROL OF NEMATODES AND SOIL-BORNE DISEASES. Wenqing Zhou, Y. Zhang, J. Byrtus, M. Samuels, C. McGregor, L. Steere, W. Cheng, T. Liu. Boragen Inc., Durham, NC 27709.

Plant parasitic nematodes are recognized as one of the greatest threats to crops around the world. Damping off infected seedlings rarely survive to produce vigorous plants. Protecting the seed and young seedling has always been crucial during early season. Boragen is a bio-tech company specialized in discovering and developing boron-based molecules with novel modes of action in nematode management and fungal disease control. Our objective has been to discover boron-based molecules with dual action against plant parasitic nematodes and fungal pathogens. To do so, we used high-throughput *in vitro* screening assays and greenhouse studies against root-knot nematodes and soybean cyst nematodes to identify boron-containing molecules in Boragen's library that have nematocidal activity. In parallel, we evaluated the antifungal activity of selected boron-containing nematocidal compounds against several soil-borne fungal pathogens. To optimize compound safety profile, we also conducted toxicology studies to select compounds that give the best balance of efficacy and safety. The results of the efficacy and safety studies will be discussed.

INTEGRATED NEMATODE AND SOIL HEALTH MANAGEMENT IN THE WESTERN HIGHLANDS OF GUATEMALAN POTATO PRODUCTION SOILS: III – ENHANCING TECHNICAL EFFICIENCY OF GROWERS. **Widanage, Rupananda<sup>1</sup>, C. Chan<sup>1</sup>, A. Mejia<sup>2</sup>, Amilcar Sanchez<sup>2</sup>, B. S. Sipes<sup>1</sup>, A. Sacbaja<sup>2</sup>, and H. Melakeberhan<sup>3</sup>**. <sup>1</sup>University of Hawaii at Manoa, Honolulu, HI 96822 USA. <sup>2</sup>Faculty of Agronomy, University of San Carlos, Guatemala City, Guatemala. <sup>3</sup>Department of Horticulture, Michigan State University, East Lansing, MI 48824 USA.

As part of the USAID's Horticulture Innovation Laboratory goal to alleviate nutrition and food insecurity, this collaborative research was conducted in smallholder potato production areas in Huehuetenango and Xela in the Highlands of Guatemala. These regions have soil health and plant-parasitic nematode management problems. In addition, the technical efficiency of the smallholder growers within and across regions of potato production is low. This paper estimates a stochastic frontier production function for four locations in Western Guatemala and attempts to identify the factors that determine technical inefficiency. The estimated model shows that technical inefficiency contributes to the 58% of production variability among potato farmers. The average technical efficiency is at 42%. In addition, there is a considerable variation in technical efficiency between four locations. Hence, there is a considerable room for the implementation of best agricultural practices for potato farming system in Western Guatemala. The estimated inefficiency model indicates that gender is a significant factor, which contributes to increase technical efficiency. This finding has vital policy implications for formulating an innovative rural development strategy which integrates gender into rural development planning in Guatemala. Likewise, this paper suggests that an increase in technical efficiency leads to improve productivity in potato cultivation and thereby increases farm household income. Thus, the findings provide policy insights for formulating a rural development strategy which, enhances productivity through improving technical efficiency in potato farmers.

CAN THE FUNGUS *FUSARIUM SOLANI* MODULATE TROPHIC CASCADES INVOLVING ENTOMOPATHOGENIC NEMA-TODES AND INSECT HERBIVORES? **Wu, Sheng-Yen1**<sup>1</sup>, **F. E. El-Borai**<sup>2</sup>, **J. H. Graham**<sup>1</sup>, **and L. W. Duncan**<sup>1</sup>. 'Citrus Research and Ed-

ucation Center, University of Florida, Lake Alfred, FL, USA, <sup>2</sup>Gulf Coast Research and Education Center, University of Florida, FL, USA. Olfactory signals are critical for soil inhabitants to communicate and respond to the environment. In addition to the well-documented volatile cues from herbivores and herbivore-induced plants, the attraction of entomopathogenic nematodes (EPNs) to their insect prey can also emanate from other sources. Using headspace solid-phase microextraction (HS-SPME) combined with gas chromatography mass spectrometry (GC-MS) analysis, two insect-attracting volatile organic compounds (VOCs) were detected from the insect-parasitic fungus *Fusarium solani*. Bioassays showed that both compounds attract entomopathogenic nematodes (EPNs) on a species-specific basis. In twochoice olfactometers, more *Steinernema diaprepesi* infective juveniles were attracted to both VOCs, whereas the closely related and often sympatric *S. khuongi* was not. Attraction to the VOCs was greater in saturated soil than in soil at field capacity. Although both compounds are highly attractive to fungivorous insects such as fruit flies, fungus gnats and rove beetles, the non-fungivorous *Diaprepes abbreviatus* root weevil was strongly repelled by one compound and unresponsive to the other. EPNs may have evolved to exploit these VOCs as a means of encountering fungivorous insects. Non-fungivorous arthropods may have evolved to avoid the compounds. These compounds have the potential of serving as baits to facilitate host finding of fungivorous arthropod pests by EPNs, especially in poorly drained soils where such pests may be prevalent.