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# ABSTRACTS

CHEMICAL DEHYDRATION OF NEMATODES FOR SCANNING ELECTRON MICROSCOPY OBSERVATIONS. **Abdel-Rahman, Fawzia H.** Department of Biology, Texas Southern University. Houston, TX 77004.

Scanning Electron Microscopy (SEM) of nematodes has become an essential component of new species description; however, the general preparation procedures for SEM can frequently result in specimen distortion during fixation and drying procedures. Here, a chemical preparation method using hexamethyldisilazane (HMDS) was employed to dry nematodes for SEM. The plant parasitic nematode *Globodera* sp., and the soil free-living nematode *Caenorhabditis elegans* were dried without any deformities. *Cyst stages of Globodera, and the* synchronized *C. elegans* hermaphrodites were used in this test. *Globodera* cysts were obtained in FAA, and the synchronized *C. elegans* hermaphrodites were killed with gentile heat, and fixed in FAA for 48 hours. All nematode specimens were ultrasonicated for few minutes and dehydrated through an acetone series, 25, 50, 75, 95%, and 3 changes of 100% acetone (30 minutes for each change). Nematodes then were transferred gradually to 100% hexamethyldisilazane (HMDS). This was done by transferring the acetone dehydrated nematodes gradually through a series of mixtures of acetone and HMDS to 100% HMDS (75% acetone and 25% HMDS, then 50% acetone and 50% HMDS, then 25% acetone and 75% HMDS, and finally 3 changes of 100% HMDS). All nematode specimens in 100% HMDS were left over night under the hood to dry. After the evaporation of HMDS, nematode specimens were observed with the stereomicroscope; dried nematodes were mounted on SEM aluminum stubs, sputter coated with gold and viewed with SEM using accelerated voltage of 15 KV. SEM Images of *C. elegans* and the cysts of *Globodera* sp., proved that drying of nematodes using HMDS were as good/or better than those regularly dried by critical-point dryer. Using HMDS to prepare nematodes for SEM is efficient, safe and easy to use.

EFFICACY AND YIELD BENEFITS OF NEMATICIDE, INSECTICIDE, AND FUNGICIDE CHEMISTRIES AND PREMIXES FOR PEST MANAGEMENT IN PEANUT. **Ahmed, Saleh, S.V. Taylor, D.A. Herbert, S. Malone, L. Byrd-Masters, and H.L. Mehl.** Virginia Tech Tidewater AREC, 6321 Holland Rd, Suffolk, VA 23437

Management of plant parasitic nematodes and other yield-limiting pests including thrips and fungal diseases is critical for maximizing yields in peanut production. Several products are available for chemical control of these pests; however, it may be cost-effective to apply these products only if pest pressure is high in a field. The objective of this study was to evaluate the efficacy and yield benefits of nematicide (fluopyram), insecticide (imidacloprid), and fungicide (prothioconazole) chemistries applied in-furrow at planting for peanuts in southeastern Virginia. A major focus of this study was to determine if and when the nematicide fluopyram provides a yield benefit in peanut, so experiments were conducted at three locations varying in nematode pressure. In-furrow treatments consisted of an untreated control, Admire Pro (imidacloprid), Velum Total (imidacloprid + fluopyram), Proline 480SC (prothioconazole), and Propulse (fluopyram + prothioconazole). For each product, equivalent amounts of active ingredients were applied so that direct comparisons can be made among treatments. In addition to in-furrow treatments, Propulse and Proline were applied at pegging in combination with either Admire Pro or Velum Total. Treatments were applied in a randomized complete block design with four to six replicates at three different locations. Thrips damage, soilborne disease incidence, soil populations of plant parasitic nematodes, and peanut yields were evaluated. Treatments including an insecticide (Admire Pro and Velum Total) reduced thrips damage across all three locations. Overall, disease pressure was low until just prior to harvest, and there were no significant differences in disease incidence among treatments. Nematode populations varied among the three experiment locations. Location one had moderate ring nematode pressure, location two had moderate root knot and high ring nematode pressure, and location three had low nematode pressure. Nematode densities in the soil increased over the growing season, but mid- and late-season nematode counts did not vary among treatments at any of the locations. At locations one and two, all treatments resulted in higher yields compared to the untreated control. The greatest increase in yield occurred in the treatment with Admire Pro applied in-furrow followed by a broadcast application of Proline at pegging. Location three had significantly lower yields compared to the other two locations, and some treated plots yielded lower than the untreated control. The in-furrow Proline treatment resulted in the highest yield at this location. Overall, there was little benefit to in-furrow applications of Velum Total in these experiments. However, nematode pressure was relatively low, and Velum Total is more likely to result in a yield response in fields with high nematode pressure. Fungicide-containing products (Proline, Propulse) provided a more consistent yield benefit across locations, suggesting fungal diseases limited yield more than nematodes. Future studies will evaluate Velum Total and other in-furrow products for peanut pest management in fields with high nematode pressure.

FROM GENES TO BIOLOGICAL CONTROL: A HIGH-THROUGHPUT SEQUENCING APPROACH TO IDENTIFYING POTENTIAL NEMATODE SUPPRESSIVE-SOIL MICROBIAL COMMUNITIES. **Alake, Gideon[1,2], P. Timper[3], D.L. Wright[4], L.W. Duncan[5], W.T. Crow[1], H.T. Alborn[6], T. Mekete[1]**. [1]Dept. of Entomology and Nematology, University of Florida, FL 32611. [2]Dept. of Crop, Soil and Pest Management, Federal University of Technology, PMB 704, Akure, Ondo State, Nigeria. [3]USDA ARS, P.O. Box 748, Tifton, GA 31793. [4]Dept. of Agronomy, North Florida Research and Education Center, University of Florida, Quincy, FL 32351. [5]Entomology and Nematology Dept., Citrus Research and Education Center, University of Florida, Lake Alfred, FL 33850. [6]USDA ARS, 1600/1700 Southwest 23rd Drive, Gainesville, FL 32608.

Plant-parasitic nematodes are of great concern in global agriculture due to the significant damage they cause to crops and the increasing management costs. Soil is a reservoir of microbial organisms with potentials for application as biological control agents, and new DNA sequencing technologies have made it progressively attractive to screen the microbiological genomic resource in soil. Metagenomic analyses of the soil microbial communities in peanut-producing counties in Northwestern and North Central Florida was conducted by sequencing the bacterial 16S ribosomal RNA and fungal internal transcribed spacer (ITS2) marker genes. The metagenomic DNA was extracted from soil samples and amplified with universal primers targeting the hypervariable regions 4 and 5 (V4-V5) of the 16S rRNA and the fungal ITS2. Here we report genomic information we garnered from analyses of intra- and inter-field soil microbial community diversities, their relative abundances as well as their functional significance. We demonstrate that a high-throughput DNA sequencing approach coupled with appropriate bioinformatic tools can generate relevant synthesis of genomic data into a biologically meaningful form. This work will help the development of a genome-centric predictive framework for understanding and harnessing of potential beneficial soil biota for management of plant-parasitic nematodes.

ABILITY OF SAR -SAPONIN AND A BACTERIAL METABOLITE TO REDUCE THE SOYBEAN CYST NEMATODE (HETERODERA GLYCINES) AND THE INCIDENCE OF THE SUDDEN DEATH SYNDROME (FUSARIUM VIRGULIFORME) **Aljaafri, Weasam Adnan[1], G.W. Lawrence[1], Shien Lu[1], V.P. Klink[2], D.H. Long[3], and K.S. Lawrence[4].** [1]Department of Biochemistry, Molecular Biology, Entomology & Plant Pathology. [2]Department of Biological Sciences, Mississippi State University, Mississippi State, MS, 39762, [3]Albaugh, LLC, 4060 Dawkins Farm Drive, Olive Branch, MS, 38654.[4]Department of Entomology and Plant Pathology, Auburn University, Auburn, AL, 36849.

Experiments were conducted to examine the ability of biological seed treatments to reduce the Soybean Cyst Nematode and the incidence of Sudden Death Syndrome. Biological seed treatments included SAR-Saponin, Bacterial metabolite, fungicides, and untreated seeds as control treatments. These seed treatments were used in test that included seed with no treatment, *H. glycines* alone*, Fusarium virguliforme* alone*, and H. glycines + F. virguliforme*. Seed applied products were received from and treated by Albaugh, LLC. Seeds were planted in 500 cm3 of a steam sterilized sand: soil mix (1:1/ V: V) in 10 cm dia clay pots, and placed in a 2.54 cm depression in each pot with 2500 eggs of *H. glycines,* and 1g of *F. virguliforme*. Tests included the standards Abamectin and Fluopyram. Treatments were arranged in a randomized complete block design with five replications. Parameter measured included effects on plant growth, nematode life stage development and the incidence of SDS. At 60 days, the biological seed treatments produced no negative plant growth effects. Treatments that included bacterial metabolite and SAR-Saponin significantly reduced SCN cyst, juveniles, and eggs compared to the control. SAR-Saponin, and bacterial metabolite were statistically similar to the standards abamectin and fluopyram. SDS foliar disease was more severe in the treatments that included *H. glycines* compared with *F. virguliforme* than *F. virguliforme* alone. SDS foliar disease index reduced from 4.4 in the control to 0.8 and 1.2 in the bacterial metabolite and SAR-Saponin treatments respectively. Bacterial metabolite and SAR-Saponin have shown potential for Sudden Death Syndrome and soybean cyst nematode management.

HOST DEFENSE SUPPRESSION MEDIATED BY THE NOVEL GR29D09 EFFECTOR FAMILY FROM THE POTATO CYST NEMATODE *GLOBODERA ROSTOCHIENSIS*. **Athena Yi-Chun, Yeh[1], S. Chen[1], T. Tran[1], and X. Wang[1,2].** [1]Cornell University, School of Integrative Plant Science, Plant Pathology & Plant-Microbe Biology Section, Ithaca, NY 14853, [2]USDA-ARS, Robert W. Holley Center for Agriculture and Health, Ithaca, NY 14853.

The potato cyst nematodes (PCN) *Globodera rostochiensis* and *G. pallida* are internationally recognized quarantine pests and considered as the most devastating pests of potatoes that can cause significant yield reduction when not controlled. The persistent presence of *G. rostochiensis* in New York and the recent detection of *G. pallida* in Idaho have adversely impacted the U.S. potato industry. Along the course of parasitism, PCN secrets a repertoire of effector proteins into root cells to promote successful infection. Among this effector repertoire, a novel family of 29D09 effectors is identified in both PCN genomes. We have cloned *Gr29D09* genes encoding four variants of the Gr29D09 effectors from *G. rostochiensis* and conducted detailed functional characterization. Using agrobacterium-mediated transient expression assays in *Nicotiana benthamiana*, Gr29D09 effectors were found to suppress flg22-triggered ROS production and defense gene expression as well as cell death triggered by a couple of resistance genes. The results clearly indicate a role for this effector family in host defense suppression. Identifying host interacting proteins may reveal the molecular mechanism of defense suppression by the Gr29D09 effectors. By utilizing ectopic expression of *Gr29D09* in potato coupled with mass spectrometry (MS) analysis, we

obtained a list of host interacting proteins. Hexokinase 7, an enzyme with a role in metabolism and sugar sensing and signaling, appears to be a strong interacting candidate of Gr29D09 as it was detected in multiple biological repeats of the MS analysis. Currently, we are using *in planta* interaction assays, including co-immunoprecipitation and bimolecular fluorescence complementation, to confirm the host targets of Gr29D09 effectors. Identifying host targets of Gr29D09 effectors likely leads to the discovery of host defense pathways that are regulated by the 29D09 effector family, which knowledge may be applicable to the development of novel nematode control strategies.

BIOLOGICAL DISCOVERIES FOR PLANT-PARASITIC NEMATODE CONTROL. **Ave-Lallemant Jr., Timothy.** AgBiome 104 TW Alexander Dr, Building 1, Research Triangle Park, NC 27709.

Plant-parasitic nematodes continue to be one of the most destructive parasites among a number of the world's largest agricultural crops. For many years, chemical fumigants like methyl bromide have been a predominate way to control plant-parasitic nematode infestations through soil fumigation. On average, plant-parasitic nematodes cause a 10%–14% yield loss annually, resulting in an $85 billion dollar loss worldwide and an $8 billion dollar loss in the United States alone. Because of concerns regarding environmental health and safety, the tools available to farmers to combat plant parasites have been limited, thus creating a need for cutting-edge innovations to control these agronomically devastating pests. At AgBiome, our goal is to develop biological solutions that will greatly reduce or eliminate the impact on yield caused by plant-parasitic nematodes. Our innovative platform, GENESIS, allows for the discovery of new biological and trait solutions for agriculture. We currently have amassed the largest fully sequenced microbe collection, consisting of over 40,000 proprietary strains collected from across the United States. AgBiome's innovative approach to nematicidal discoveries involve high-throughput bioassays to identify potential actives against plant-parasitic nematodes. By using integrated screening pipelines, we have identified novel active strains with in vitro nematicidal activity. These candidate biological control strains will be tested by using on-plant and field screening formats in 2017.

DEVELOPING A REAL-TIME PCR ASSAY FOR DIRECT IDENTIFICATION AND QUANTIFICATION OF SOYBEAN CYST NEMATODE, *HETERODERA GLYCINES*, IN SOIL. **Baidoo, Richard and G.P. Yan.** North Dakota State University, Department of Plant Pathology, Fargo, ND 58108.

The soybean cyst nematode (SCN), *Heterodera glycines*, is a major threat to soybean production world-wide. Morphologically, it is hard to differentiate SCN from other members of *H. schachtii sensu stricto* group. The task is even more complicated when quantifying SCN by eggs which are almost morphologically inseparable from other nematode eggs. This problem may result in false positive or negative report and over- or under-estimation of numbers especially when SCN population density is low in a mixed population with other nematode species. Moreover, conventional cyst and egg extraction methods are not only time-consuming but labor and cost intensive. A SYBR Green I-based quantitative real-time PCR (qPCR) assay was developed to identify and quantify SCN directly in DNA extracts of field soils, and to differentiate SCN from morphologically closely related species such as *H. schachtii*, *H. trifolii*, *H. ciceri*, and *H. avenae* which may occur in North Dakota. SCN-specific qPCR primers were designed from a putative parasitism gene, *CLAVATA3*, which showed high specificity to SCN and consistently produced a single amplicon in melting curve analysis. The specificity of the assay was also evaluated using seven isolates of SCN and 31 other nematode species. Varying numbers of SCN eggs or juveniles (0, 1, 4, 16, 64, 256) were inoculated into 0.25 g sterilized soil from which soil DNA was extracted using the DNeasy PowerSoil® Kit and a standard curve relating threshold cycle and log values of nematode number was generated ($y = -3.41x + 31.16$; $E = 96.4$ %; and $R^2 = 0.96$). The assay was validated by quantifying different SCN numbers artificially added to a sterilized soil before it was used to estimate SCN numbers in 32 field soil samples naturally infested with this nematode at varying levels. The identities of *H. glycines* in the field soils were confirmed by randomly sequencing of two genomic regions (D2-D3 of 28S rRNA and ITS rDNA) of 15 populations. For each soil sample, 400 g of soil was collected and divided in half for molecular quantification, and traditional egg extraction and microscopic enumeration. There was a high correlation between the SCN numbers quantified by the qPCR assay and the conventional method in both artificially and naturally infested soils. Grinding the field soil prior to DNA extraction improved SCN detection and quantification efficiencies. The assay will not only be useful for simultaneous identification and quantification of SCN eggs and juveniles directly from soil, but also to differentiate SCN from closely related species. It requires no expertise in nematode taxonomy or morphology and circumvents the time-consuming steps of cyst extraction and crushing, sieving and decanting, and microscopic identification and counting. Accurate identification and quantification of SCN prior to planting are essential for developing effective integrated pest control measures and yield-loss risk assessment.

TRANSCRIPTOME APPROACH TO IDENTIFY GENOME WIDE EFFECTS OF SILENCING TWO ESOPHAGEAL GLAND GENES OF *MELOIDOGYNE INCOGNITA*. **Banakar, Prakash, and U. Rao.** Division of Nematology, ICAR-Indian Agricultural Research Institute, New Delhi-110012, INDIA.

Root-knot nematode, *Meloidogyne incognita* is one of the most economically important plant parasitic nematodes worldwide, with a host range more than 3000 plant species. RNA interference (RNAi) or post transcriptional gene silencing

(PTGS) has been found to be of immense value for deciphering the gene function by way of knocking down the genes particularly in the obligate plant parasitic nematodes. Present study was carried out to identify the oscillating effects of silencing two effector genes on global transcriptome of *M. incognita* second stage juveniles (J2s) using RNA seq approach. The two esophageal gland specific genes, *msp-1* and *msp-20*, were silenced individually by soaking in respective dsRNA. Total RNA from both control and silenced nematodes were used for transcriptome sequencing using Illumina platform. Deep sequencing of transcriptome resulted in about 14 million high quality reads with an average GC% of 37.83% for each of the sample. Merged assembly of HQ reads generated 1,02,438 non-redundant transcripts. All the expressed transcripts were subjected to BLASTx annotation with an eValue cutoff <0.001 against predicted *M.incognita* proteins, Wormbase, Nembase, UNIPROT and NrDB (whole non-redundant Protein Database). A total 80,547 out of 1,02438 expressed transcripts could be annotated by using one or more of the above databases. Differential gene expression of the expressed transcripts was performed using DESeq software based on R programming environment. Expression profiling of transcripts assembled in silenced and control libraries revealed a significant degree of baseline expression and also very specific siRNA induced gene expression changes. We observed that considerable portion (1,913/2,364) of gene expression changes induced by gene silencing were common between *msp-1* and *msp-20*. Further, 441 transcripts were expressed uniquely in *msp-20* silenced nematodes, while only 10 were specific to *msp-1* dsRNA treated nematodes, indicating significant perturbation by *msp-20* dsRNA. Baseline expressed transcripts were again subjected to differential gene expression profiling by comparing *msp-20* dsRNA treated vs control and *msp-1* dsRNA treated vs control at a detection stringency of 2 fold up or down regulation with a pValue of <=0.05 (indicative of 5% False Discovery Rate). This revealed up regulation of 2698 transcripts while, 1783 genes were down regulated in *msp-20* silenced nematodes. On the other hand, *msp-1* silencing led to the up regulation of 266 transcripts and down regulated 402 transcripts. Interestingly, distribution analysis of up and down regulated transcripts showed very less number of transcripts to be commonly up or down regulated between both the treatments demonstrating a strong gene regulatory control of both the effectors on the genome wide transcriptome of *M. incognita*.

FUNCTIONAL CHARACTERIZATION OF *MI-TRA-1* AND *MI-SDC-1* IN SEX DETERMINATION OF *MELOIDOGYNE INCOGNITA*. **Baniya, Anil[1], S. Joseph[1], L.W. Duncan[2], W.T. Crow[1], T.M. Mekete[1].** [1]Entomology and Nematology Department, University of Florida, FL 32611. [2]Citrus Research and Education Center, University of Florida, Lake Alfred, FL 33850.

Root-knot nematodes (*Meloidogyne* spp.) are among the most destructive and widespread plant-parasitic nematodes in the world with extremely wide host and geographical ranges. *Meloidogyne incognita* has mitotic parthenogenetic mode of reproduction, with males and females. Females are sedentary and induce galls whereas, males move freely without significant infection. The molecular process of sex determination in this nematode has not been well studied. Proper understanding of sex determination pathways could be an effective tool for developing sustainable management strategies against this nematode. In *Caenorhabditis elegans, the sdc-1* gene has been shown to be the upstream regulator of the female sex determination cascade where *tra-1* is a downstream regulator of the pathway. The knock down of these two genes in *C. elegans* results in defects in embryonic development and egg laying in females. The orthologs of these genes have been identified in *M. incognita*. The aim of this study was to determine the possible function of the *Mi-tra-1* and *Mi-sdc-1*in sex determination of *M. incognita*. RNAi was performed by soaking second-stage juveniles of *M. incognita* in a solution containing dsRNA of either *Mi-tra-1*or *Mi-sdc-1* for 24hr. The levels of both *Mi-tra-1* and *Mi-sdc-1* mRNAs were significantly reduced in a sequence-specific manner in nematodes soaked for 24h. The downregulation of both genes was observed 4 days after recovering the juveniles from the dsRNA treatment. Neither the treatment with dsRNA of *Mi-tra-1* nor with the dsRNA of *Mi-sdc-1* resulted in sex reversal to male nematodes. However, RNAi of *Mi-sdc-1*-significantly delayed the maturity of females, whereas the downregulation of *Mi-tra-1* showed significant impact on female fecundity as compared to the treatment with dsRNA of GFP and untreated control.

INSIGHTS INTO EPN SPECIES INTERACTIONS FROM THE GENOMES OF THEIR MICROBIAL SYMBIONTS. **Bashey, Farrah, M. Gaughan, D. Rusch, and T. Doak.** Department of Biology, Indiana University, 1001 E. 3rd Street, Bloomington IN 47405.

Field surveys have shown that multiple *Steinernema* species can be isolated from the same locale and often utilize the same insect host species. Species differences in movement patterns, host preferences, environmental tolerances, and life-history patterns can help shape our understanding of how these nematodes partition the EPN niche. Additionally, variability in competitive interactions due to priority effects and genotypic-specific interactions can further help to maintain multiple species. We have found that bacteriocins produced by *Xenorhabdus* symbionts of *Steinernema* can kill other sympatric *Xenorhabdus* isolates, thereby preventing the success of their nematode associates. However, the competitive success of nematodes carrying bacteriocin producers depends on the presence of nematodes carrying symbionts sensitive to their bacteriocin. In the absence of a sensitive competitor, earlier arriving or faster developing nematodes prevail. Thus, a dominant species in one interaction may be competitively inferior in the next, thereby promoting coexistence. Further, phenotypic analyses show multiple bacteriocin phenotypes within each nematode species, suggesting that intraspecific

diversity may be important in maintaining species diversity. Here we present genomic analyses of 80 isolates of two species of *Xenorhabdus* bacteria collected from four nematodes species found in three natural communities. We examine the evolutionary history of these bacterial isolates using gene presence/absence analysis and nucleotide comparison of conserved protein coding regions. We also analyze the bacteriocin regions to determine the genetic basis for the observed phenotypic diversity. These genomic analyses support the dynamic nature of nematode competition mediated by their microbial symbionts.

TWO RESISTANCE QTLS IN COTTON TO MELODOGYNE INCOGNITA HAVE DIFFERENT EFFECTS ON EGG PRODUCTION. **Batista da Silva, Mychele[1,2], P. Kumar[2], R. F. Davis[3], R.L. Nichols[4], and P.W. Chee[2].** [1]Department of Plant Pathology, University of Georgia, Tifton Campus, Georgia, [2]Cotton Molecular Breeding Laboratory, University of Georgia, Tifton, Georgia, [3]USDA-ARS Crop Protection and Management Research Unit, Tifton, Georgia, [4]Cotton Incorporated, Cary, North Carolina.

Cotton is widely grown in the southern US and *Meloidogyne incognita* is the most significant pathogen of cotton in the US. Resistance to *M. incognita* is available and is the most cost effective management strategy. M-120 RNR (M-120) germplasm is highly resistant to *M. incognita* due to two resistance QTLs, one on chromosome 11 (*qMi-C11*) and one on chromosome 14 (*qMi-C14*). Previous research showed that both QTLs reduce total egg production but the QTLs affect *M. incognita* development differently: *qMi-C11* interferes with feeding site establishment or gall development which stops many nematodes from developing beyond the J2 or SJ2 stage, whereas *qMi-C14* does not interfere with feeding site establishment or gall development but prevents late stage juveniles (J3/J4) from developing into females. Because one QTL is affecting the nematode during gall development, but the other is not, we hypothesized that gall size may be differentially affected by the QTLs. We conducted an experiment with two trials to measure root gall size using isogenic lines containing both QTLs (M-120), only one QTL (C11 with *qMI-C11* or C14 with *qMi-C14*), or neither QTL (C201). Individual seedlings were planted in clear plastic bags containing vermiculite and inoculated with *M. incognita*. When galls first appeared (day 0) individual galls were labeled and each gall's size was measured at 0, 7, and 14 days. No consistent differences in gall size were observed. We also evaluated whether the QTLs contributed to resistance by reducing the number of eggs per egg mass. We conducted an experiment with three trials to measure the number of eggs per egg mass. We harvested 10 egg masses/plant/genotype (7 replications) at 30 and 40 days after inoculation (DAI). At 30 DAI, just one trial showed significant differences, but many egg masses had not yet reached their final size. At 40 DAI, C201 had more eggs per egg mass than M-120 in all trials, whereas C11 and C14 typically were numerically intermediate. C14 had similar eggs per egg mass to M-120 and C11 was similar to C201 in all trials. Other differences were less consistent with C14 having fewer eggs per egg mass than C201 in trials 2 and 3, C14 having fewer than C11 in trial 2, and C11 having more than M-120 in trial 2. We conclude that 1) *qMi-C14* causes a reduction in the number of eggs per egg mass whereas *qMi-C11* does not, and 2) neither *qMi-C11* nor *qMi-C14* affect gall size.

PROTECTION OF FRESH MARKET CARROTS AGAINST ROOT-KNOT NEMATODE-CAUSED DAMAGE WITH NOVEL NEMATICIDES. **Becker, J. Ole[1], A. Ploeg[1], and J. Nunez[2].** [1]Department of Nematology, University of California, Riverside, CA 92521, and [2]University of California Cooperative Extension Kern County, Bakersfield, CA 93307.

During the past decade, California has produced near 90% of US fresh market carrots on approximately 28,000 hectares. Root-knot nematodes, particularly *Meloidogyne incognita* and *M. javanica* induce root galling and forking as well as reduce crop yield due to diminished water and nutrient uptake. As such, they are the primary disease constraints in California's carrot production. The pathogens' large host range, the absence of commercial carrot cultivars with root-knot nematode resistance, and the unavailability of potent contact nematicides, biological or biorational control products have perpetuated the use of pre-plant soil fumigants. In collaboration with plant protection companies and the California Fresh Market Carrot Advisory Board, our group has assessed novel nematicides and their application methods for efficacy in *M. incognita*-infested field trials. The development products Nimitz (a.i. fluensulfone, Adama) and Salibro (a.i. fluazaindolizine, DuPont) greatly reduced disease symptoms under high disease pressure caused by the Southern root-knot nematode. They increased marketable carrot yield from 60 to 90%, respectively, compared to about 10% in the non-treated control. In addition to their excellent efficacy, these novel nematicides distinguish themselves from earlier generations of nematicides by much lower mammalian toxicities and environmentally safer profiles.

NEMATODE ASSEMBLAGES AND SUCCESSION ON AND UNDER BEAVER CARCASSES. **Bernard, Ernest C.[1], G. Phillips[1], L.S. Taylor[2], S.W. Keenan[2] and J.M. DeBruyn[2]**. [1]Entomology & Plant Pathology, [2]Biosystems Engineering & Soil Science, University of Tennessee, Knoxville, TN 37996 USA.

Vertebrate carcass decomposition provides abundant but ephemeral sources of enrichment that should be well-suited to bacterivorous CP-1 nematodes, which have short life cycles and high fecundity. This idea was tested in a field experiment using North American beaver carcasses placed in a medium-age mixed hardwood forest at the University of Tennessee Arboretum. Six beavers, each approximately 20 kg and delivered frozen, were placed individually in large wire-mesh cages placed directly on the forest floor. Soil under beavers and in control plots 2 m from each cage were assayed before carcass

placement (preplacement) and at successive stages of decomposition: fresh (2 days after placement), bloat (6 days), active decomposition (15 days), advanced decomposition I (35 days) and advanced decomposition II (40 days). Plates of NGM agar inoculated with *E. coli* OP50 were exposed to extra preplacement soil (12 samples) to isolate and culture naturally occurring bacterivorous nematodes. In the latter three samplings nematodes also were collected from interface soil (surface soil in direct contact with the carcass) by scraping the soil surface. In addition, interface-like samples were collected from control plots 40 days after placement in order to compare their nematofaunas with interface samples from cages. For soil cores and interface samples nematodes were extracted from 100 $cm^3$ soil. Insects arriving at carcasses were collected live and placed singly into agar dishes for development of phoretic nematodes. Tissue samples were collected from carcasses 15 days after placement and placed on agar for culture of nematodes infesting the tissue. Nematodes were identified by means of a combined morphological-molecular approach largely to genus. Bacteria-feeding nematodes from preplacement samples were primarily *Acrobeloides*, *Oscheius* and *Rhabditis* spp. and Diplogasteridae. Nematodes were not isolated from any of the 60 assayed Diptera in the families Calliphoridae, Drosophilidae, Muscidae, Piophilidae and Sarcophagidae. However, nearly all of the 50 specimens (three species) of Silphidae (Coleoptera) collected on or near carcasses were infested with *Rhabditoides inermis* carried phoretically under the elytra. Only one of 14 tissue samples contained nematodes (*R. inermis*); this sample originated from a part of the beaver in contact with the soil surface. Nematode assemblages in control and cage soil cores were generally similar for the first 15 days, with *Filenchus* and *Helicotylenchus* spp. codominant. In later samplings, rhabditids were the most numerous taxon due to their high numbers at the surface. Cage interface samples were dominated by rhabditid nematodes (up to 24,000/100 $cm^3$ soil), primarily *Rhabditella* sp., *Pelodera cylindrica* and *Pelodera* sp.; these nematodes were not observed in control plots. Control interface-like samples had species compositions similar to those of control plots. Beaver decomposition stimulated soil microfauna and provided an abundant food source for bacterivorous nematodes. The colonization of soil by presumed insect-derived phoretic nematodes demonstrates the importance of decaying carcasses to broader ecosystem functioning.

DIVERSITY, PHYLOGENY, CHARACTERIZATION AND IDENTIFICATION OF NEMATODES: THE GHENT UNIVERSITY STRATEGY. **Bert, Wim, X. Qing, Y.A. Kolombia, D. Slos, M. Couvreur, T. Janssen.** Nematology Research Unit, Department of Biology, Ghent University, K.L. Ledeganckstraat 35, 9000 Ghent Belgium.

Free-living nematodes are important as bio-indicators for environmental monitoring and plant-parasitic nematodes are significant pests in crop production. However, the characterisation and identification of nematodes is greatly hampered by phenotypic plasticity, interspecific similarities and the existence of cryptic species complexes. Furthermore, the vast majority of morphospecies are not linked to DNA sequences. We describe our strategy to target a comprehensive and reliable description of nematode biodiversity for the plant-parasitic genera *Meloidogyne*, *Pratylenchus* and *Scutellonema* and for some non-parasitic nematodes (Tylenchidae, Sphaerularioidea and Rhabditidae). For *Meloidogyne*, nine quickly-evolving mitochondrial coding genes were screened to determine a suitable barcode region. Nucleotide polymorphisms harbor enough variation to distinguish these closely-related lineages, and completeness of lineage sorting was verified by screening 80 populations from widespread geographical origins and various hosts. These mitochondrial haplotypes are strongly linked and consistent with traditional esterase isozyme patterns, indicating that different parthenogenetic lineages can be identified reliably using mitochondrial barcodes. Multi-gene phylogenetic analysis in combination with cytogenetics of basal species revealed multiple independent origins of mitotic parthenogenesis. For *Pratylenchus* a combination of a multi-gene phylogeny in combination with molecular species delineation, morphometrics, ecological information and sequences from type location material allowed us to clarify long-standing debates about the taxonomic status of several *Pratylenchus* species; *P. penetrans, P. fallax, P. convallariae P. brachyurus, P. pinguicaudatus, P. dunensis, P. oleae* and four new species were recognized as separate taxonomic entities with clear genetic boundaries. Our study stresses the importance to safeguard the link between DNA barcodes, morphology and sequences from topotype locations, as exemplified by the high number of misidentified species on GenBank. For *Scutellonema*, molecular phylogeny, molecular species delimitation and morphology revealed an undiscovered diversity of *Scutellonema* species associated with yam. However, only *S. bradys* was identified from yam tuber tissue. For both *Pratylenchus* and *Scutellonema* DNA barcoding was established as a valuable alternative for species diagnostics, the *COI* gene being the most reliable marker. Finally, our integrative approaches are further illustrated for free-living nematodes, including the use of informative ultrastructural morphology, genomic data, population genetics, comprehensive databanks and 3D-printing.

FREE-LIVING NEMATODES IN MARINE SEDIMENTS: LINKING MOLECULES WITH MORPHOLOGY IN THE -OMICS AGE. **Bik, Holly M**. Department of Nematology, University of California—Riverside, Riverside, CA 92521, USA.

Free-living nematodes are ubiquitous and numerically abundant in marine sediments, often representing 85-96% of the total meiofauna community. Yet, we still lack an overall understanding of the global patterns of biodiversity and biogeography for most marine nematodes. Even less well understood is the relationship between nematode species and prokaryotic microbes, such as bacterial symbioses and predator-prey interactions. To address this knowledge deficit, Environmental 'Omics tools are being increasingly used to address major gaps in our knowledge of diverse free-living nematode groups. ''Molecular taxonomy'' encompasses a number of distinct approaches, including single-specimen DNA barcoding, environmental rRNA surveys, and targeted genome sequencing. This talk will discuss recent work using parallel high-throughput

sequencing and taxonomic approaches to explore broad patterns in free-living marine nematode assemblages (biodiversity and phylogeography, functional roles for microbial taxa, and the relationship between species and environmental parameters), including phylogenetic comparisons of nematode microbiomes.

ROOT KNOT NEMATODE MANIPULATES AMINO ACID BIOSYNTHESIS IN GIANT CELLS. **Bird, David[1,2], and M. Miklavcic[3].** [1]Department of Entomology and Plant Pathology, and [2]Bioinformatics Research Center, North Carolina State University, Raleigh, [3]Chemical and Life Sciences program, University of Maryland.

It is axiomatic that Giant Cells (GC) are the food source for root-knot nematode (RKN). However, other than functioning as carbohydrate sinks loaded from the phloem stream, the precise role of these unique plant cells remains obscure. Photo assimilate in the phloem is rich in sugars, but sparse in amino acids, and thus has unbalanced nutritional value. Aphids, also phloem-feeders, alter the carbon-to-nitrogen ratio (C:N) by excreting excess sugar (as honeydew), and by deploying bacterial symbionts to manufacture amino acids. Because they are physically contained within the host root, this strategy probably is not an option for RKN. Rather, we propose that GC function as factories to optimize C:N thus making the phloem stream palatable. It has been demonstrated that the feeding cells induced by cyst nematodes actively polymerize sugars into starch, which is presumed to buffer the energy needs of the parasite (Hofmann et al, *Plant Physiol*, 2005). Alternatively, sequestering the sugars into starch renders them non-toxic in the same manner that blood-feeders (such as the malarial parasite) polymerize heme to render it non-toxic. Using a genetic approach based on expression QTL mapping, Guo et al (*Genetics*, 2017) established causal relationships between host and parasite genes. Network inference revealed that defined loci in RKN play a role in regulation of plant methyl (1-carbon) and acetyl (2-carbon) transferases in the GC of their host. These enzymes lie at the heart of amino acid biosynthesis, including those unable to be made by animals. Remarkably, it was recently found that a locus in soybean, named *RHg4,* which conditions stable and effective resistance to cyst nematodes (Liu et al, *Nature*, 2012), encodes a methyl transferase. Precisely how an isoform of an amino acid biosynthesis enzyme conditions resistance is not apparent. One possibility is that it is related to the nematode's perception of food quality, and subsequent behavioral response to suspend feeding. *C. elegans* exhibits such a response to low food quality, and aphids (and also RKN) exhibit antifeedant behavior in response to the tomato *Mi* gene. Collectively, these results point to GC as being purveyors of C:N balanced food for RKN, and to RKN being discerning diners.

A GENOME PHYLOGENY OF NEMATODA. **Blaxter, Mark.** Institute of Evolutionary Biology, University of Edinburgh, Edinburgh EH9 3JT, UK.

Phylum Nematoda is speciose and diverse, and hypotheses of the relationships of different groups of nematodes are important for understanding the origins of this diversity. Two decades ago, we published the first phylum-wide phylogeny of Nematoda, based on a single marker (the nuclear small subunit ribosomal RNA gene, nSSU or 18S) from only 53 species. This phylogeny supported many relationships previously proposed on morphological grounds, but contradicted others, and raised many questions. In the last decades the analysis of nematode relationships using molecular data has blossomed, and there are now over 4000 nominal taxa for which nSSU data are available, and phylogenies encompassing over a thousand species have been published. In these new analyses, some nodes remain unresolved, and it is likely that we are at the limit of the resolving power of a single gene. The first nematode genome sequence – indeed the first genome sequence of any animal was also published two decades ago. In the last decade, the availability of high-throughput DNA sequencing has enabled the sequencing of the genomes of many more species of nematode. To date there are over 120 taxa for which genome data are publicly available. While these taxa are not evenly distributed across the diversity of Nematoda there is a strong bias towards parasites of health or agricultural importance they can be used for phylogenetic inference. By identifying large numbers of single-copy genes we have been able to build genome phylogenies of Nematoda. These confirm the core structure proposed twenty years ago, and also provide strong support for previously unresolved nodes. The genome data I will discuss has been produced by laboratories worldwide, and I thank all those researchers for their efforts.

PLANT-NEMATODE LIPIDOME: A TRANSCRIPYOMIC AND METABOLOMIC INSIGHT. **Braun Miyara, Sigal[1], N. Fitoussi[1,2], F. Gurung[1,2], P. Bucki[1], N. Naor[1,2], B. Chinnapandi[1].** [1]Department of Entomology, Nematology and Chemistry units; Agricultural Research Organization (ARO), the Volcani Center, Bet Dagan, 50250, Israel. [2]Department of Plant Pathology and Microbiology, the Faculty of Agriculture Food & Environment, the Hebrew University of Jerusalem, Rehovot, 76100, Israel.

Recent findings have shown that successful long-term relationship between the biotrophic sedentary endoparasitic root-knot nematodes (RKN), *Meloidogyne spp.,* and their hosts rely on a well programmed secretion of effector proteins. These efectors interfere with and mimic multiple physiological and morphological mechanisms, leading to modifications and reprogramming of the host cells' functions, thus enslaving the cells to complete their life cycle. In the present study we aimed to reveal novel effectors carrying an immunosuppression activity, through oxylipins, oxygenated lipids derived from polyunsaturated fatty acids, modulation. For that purpose a metabolic profiling of fatty acid derived molecules composition during *M. javanica* infection of tomato was conducted using LC-MS/MS approach. Our results indicate on identification of

oxylipin products that are specifically altered upon tomato root inoculation with the RKN, *M. javanica,* suggesting that different oxylipins are maneuvered by the nematode-secreted effectors. Moreover, RNAseq was performed and generated a total of 6335 DEGs of *M.javanica* second stage juveniles (J2s) exposed to tomato protoplast, 9-HOT and 13-KOD oxylipins. The main groups of genes that have shown to be differentially expressed include genes carrying a predicted secretion signal peptide, genes involved in cell wall degradation and hormone metabolism regulation. Among the DEGs including signal peptide, several had homology with known effectors in other nematode species, other unknown potentially secreted proteins may have a role as RKN's effectors, interacting with lipid signaling. This research will provide a better understanding of oxylipins function in regulating the outcome of the parasitic interaction.

EUPHRESCO PROJECT ON CYST AND ROOT-KNOT NEMATODE INVENTORIES FROM AROUND THE WORLD. **Bulluck, Russ and A.C. Kaye**. USA APHIS PPQ S&T Director's Office, 1730 Varsity Dr., Suite 400, Raleigh, NC 27606.

Research activities on cyst and root-knot nematodes, specifically *Meloidogyne* spp. and *Globodera* spp., take place all over the world; however, the collection, maintenance, and storage of these nematodes are costly. Because of this cost pressure, the number of living nematodes populations in global collections is decreasing. A project through the European Phytosanitary Research Coordination (EUPHRESCO) is making an inventory of *Meloidogyne* spp. and *Globodera* spp. live collections in seven different countries, including the United States, as well as the maintenance techniques used in the nematode laboratories. The ultimate purpose for this information is to form a collaborative global effort to maintain these existing populations, create a universal maintenance manual for cyst and root-knot nematodes, and to possibly make specimens available for all researchers. To help foster the exchange of rearing and maintenance information about these nematode species, a workshop will be organized in September 2017 through the European and Mediterranean Plant Protection Organization and the Dutch National Reference Center. In the United States, beginning in July 2016, the authors have contacted over 50 different researchers about the status of their institution's nematode collections. Researchers were asked to provide the following information: the full taxonomy including any subspecies designation, where and when the population was collected/obtained, and a copy of any maintenance manual/protocol being used. So far, the collected responses show that researchers are maintaining populations of *G. tabacum solanacearum*, *G. tabacum tabacum*, *M. arenaria*, *M. hapla*, *M. incognita*, *M. javanica*, *M. floridensis*, and *M. enterolobii*. During the summer of 2017, a follow-up questionnaire will be distributed back to the responding researchers to ask for further details about length of storage without loss of viability, specific hosts and cultivars for rearing, climatic rearing conditions, and watering and temperature regimes.

*BURSAPHELENCHUS ANTONIAE* FROM *PINUS STROBUS* IN THE U.S. **Carta, Lynn[1] and R.L. Wick[2].** [1]USDA-ARS Mycology and Nematology Genetic Diversity and Biology Laboratory, Beltsville Agricultural Research Center (BARC), Beltsville, MD; [2] University of Massachusetts, Amherst, MA.

Juvenile, female and male nematodes were discovered in wood chips of white pine *Pinus strobus* from Ashley Falls, MA. The white pine specimen was submitted to the UMass Nematology Lab to examine for the pine wood nematode (PWN), *Bursaphelenchus xylophilus*, as required for shipment of pine logs to China. Initial observations suggested these nematodes might be PWN, but closer morphological and molecular characterization proved otherwise. Comparison of measured features with those in the literature indicated this nematode population had some unique characteristics. The specimens were identified as *Bursaphelenchus antoniae* Penas et al., 2006 based on 18S rDNA molecular sequence (100% similar in the aligned region) vs. only 95% similarity with PWN *B. xylophilus*. Compared to the previously described Portugal population of *B. antoniae*, the sequences generated for the MA population were 98.3% similar in the ITS1,2 rDNA and 99.9% similar for 28S rDNA. A sequence for cytochrome oxidase I was generated that is unique within GenBank. This population has morphology consistent with that of Penas et al., 2006; however, the female tail on this MA *Pinus strobus* population is distinctly more attenuated than in *B. antoniae* from *P. pinaster*/*Hylobius* and has a smaller c' value.

EVALUATION OF TRICHOMAX WP (*Trichoderma asperellum*) AND KLAMIC WP (*Pochonia chlamydosporia*) FOR BIOLOGICAL CONTROL OF PLANT PARASITIC NEMATODES IN BANANA. **Castellanos, Flor and Luis Pocasangre.** EARTH University, Las Mercedes, Limón, Costa Rica.

The objective of this research was to evaluate the effect of TRICHOMAX WP (*T. asperellum*) and KLAMIC WP (*P. chlamydosporia*) in the population of plant parasitic nematodes in three banana cultivars of Cavendish subgroup: Bonifacio, Grande Naine and Williams in the commercial plantations. The research was conducted in the block one of the commercial banana farm of EARTH University. The experiment was set out in the random block design with three replicates. Three bimonthly applications were conducted of 2, 5 g per plant excluding the control, three bimonthly samplings were conducted after the application to evaluate the population of plant parasitic nematodes: *Radopholus similis, Meloidogyne incognita* and *Helicotylenchus multicinctus*. Regarding to root system, the variables evaluated were: root health index, necrotic index, weight of functional, death, total roots and diameter of the roots. The use of TRICHOMAX WP and KLAMIC WP reduced significantely the populations of all three plant parasitic nematodes no matter the cultivar, TRICHOMAX WP

the best performance recorded. Regarding to variables of root health, the best treatments in order were T5 (*Trichoderma asperellum* (E1)-Bonifacio), T3 (*Pochonia chlamydosporia*-Gran Enano) and T6 (*Pochonia chlamydosporia* (E2)-Bonifacio), there was a statistical difference detected ($p<0.05$) between the treatments. In the case of necrotic index, weight of functional, death and total roots there was not a statistical difference detected ($p<0.05$).

ACTIVATED ENTOMOPATHOGENIC NEMATODE INFECTIVE JUVENILES RELEASE LETHAL VENOM PROTEINS. **Chang, Dennis Z[1], D. Lu[1], M. Machietto[2], L. Serra[2], A. Mortazavi[2], A.R. Dillman[1]** [1]Department of Nematology, University of California Riverside, Riverside California 92521, [2]Department of Developmental and Cell Biology, University of California, Irvine, CA 92697.

Entomopathogenic nematodes (EPNs) are insect parasites used in biological pest control. Free-living, developmentally arrested infective juveniles (IJs) find and infect insects. Upon infection the IJs activate, release symbiotic bacteria, and recover from developmental arrest. IJ/bacterial complexes are efficient parasites, usually killing their host within 72 hours. Although many helminth parasites are known to produce excreted-secreted products (ESPs), the virulence of EPNs has been primarily attributed to their symbiotic bacteria. We wanted to explore the potential nematode contribution to pathogenicity against the host. Using EPNs from the genus *Steinernema*, we developed a systematic method of quantifying IJ activation and improved an *in vitro* activation model. RNA-sequencing shows high similarity between the gene expression profiles of *in vitro* and *in vivo* activated EPNs validating our *in vitro* method as a good model of *in vivo* EPN parasitism. Collected ESPs from the activated IJs exhibited high toxicity in different insects including in *D. melanogaster G. mellonella*, and *B. mori*. Mass spectrometry and bioinformatic analyses revealed over 400 proteins, many with high sequence similarity to proteins important in other parasites such as proteases and protease inhibitors. The ESPs also showed high sequence similarity to mammalian-parasitic nematodes in the genera *Strongyloides*, *Anclystoma*, *Toxocara* and others. Preliminary protein fractionation data show reduced toxicity in some fractions while other fractions showed enhanced toxicity compared to crude ESPs. This is likely due to enrichment of the active components. We are working to further isolate and identify insecticidal components. Our results provide strong evidence that entomopathogenic nematodes contribute significantly to the pathogenicity within hosts. Furthermore, the sequence similarity between the secreted proteins of EPNs and vertebrate parasites reaffirms that EPNs are good models for studying parasitic nematode biology.

ASSOCIATION MAPPING OF CHLOROPHYLL CONTENT ASSOCIATED WITH SOYBEAN CYST NEMATODE IN SOYBEAN. **Chen, Senyu[1], Jun Qin[2], Liana Nice[3], Aaron Lorenz[3], Nevin D. Young[4], and James H. Orf[3].** [1]Southern Research & Outreach Center, University of Minnesota, Waseca, MN 56093, USA; [2]Department of Horticulture, PTSC316, University of Arkansas, Fayetteville, AR 72701, USA; [3]Department of Agronomy and Plant Genetics, University of Minnesota, St Paul, MN 55108, USA; and [4]Department of Plant Pathology, University of Minnesota, St Paul, MN 55108, USA.

Soybean cyst nematode (SCN, *Heterodera glycines* Ichinohe) is the most important pathogen of soybean. One impact on soybean by SCN infection is the induction of nutrient-deficiency chlorosis, especially iron-deficiency chlorosis (IDC). The objective of this study was to conduct genome-wide association study (GWAS) and identify single nucleotide polymorphisms (SNP) markers for chlorophyll content in soybean without SCN infection and SNP markers for reduction of chlorophyll content by the infection of the SCN. A total of 194 soybean representative genotypes (lines) from the University of Minnesota soybean breeding program were used in this study, with the phenotyping conducted in the greenhouse conditions using a completely randomized design (CRD) with four replicates. Soybean was grown in pots without SCN infestation or infested with 5,000 eggs/100 $cm^3$ soil, and soybean leaf chlorophyll contents (LCC) of the second trifoliolate leaves were measured about 8 weeks after planting. Percentage reduction of LCC of a soybean line by SCN [= 100 × (LCC without SCN – LCC in soybean infected by SCN) / LCC without SCN] was calculated for individual pairs of pots. A total of 4,252 SNPs from the Soy6K SNP Infinium Chips was used as genotyping. On average, SCN reduced LCC by 19.0%. Significant IDC symptom was observed in a number of soybean lines in both SCN-infested and non-infested soil. SNP markers for LCC in non-infested soil were identified at six regions of six chromosomes: one at each of chrs. 3, 9, 11, 13, 18, and 19; and SNP markers associated with the reduction of LCC by SCN were identified at six regions of four chromosomes: one at chr. 4, one at chr. 6, two at chr. 13, and two at chr. 15. The research opens a new approach to manage SCN and nutrient-deficiency chlorosis (e.g., IDC) through breeding soybean for SCN and chlorosis tolerance, and the SNP markers will provide a tool for breeders to select the SCN and chlorosis tolerance through marker-assisted selection (MAS).

*SLWRKY45*, A MULTI- FUNCTIONAL TOMATO TRANSCRIPTION FACTOR, IS REGULATES ROOT KNOT NEMATODE *MELOIDOGYNE JAVANICA* DISEASE DEVELOPMENT IN TOMATO ROOTS. **Chinnpandi, Bharathiraja and Braun Miyara Sigal**. Department of Entomology and Units of Nematology and Chemistry, Agricultural Research Organization (ARO), The Volcani Center, Rishon Lezion, Israel.

Plant WRKY's represents a major class of zinc –finger transcription factors play an important role in diverse signaling pathways involved in biotic or abiotic stress response. Information concerning the fluctuation of WRKYs protein during plant parasitic nematode infection is limited. In the present study spatial and temporal expression of WRKY45 were studied in

depth with regards to their responses to nematode infection, phytohormones, and wounding. Expression of *WRKY45* increased substantially within 5 days and continued through feeding-site development and gall maturation. Histological analysis of nematode feeding sites indicated that *WRKY45* was highly expressed within the feeding cells and associated vascular parenchyma cells. Responses of *SlWRKY45* promoters to several phytohormones showed that *WRKY45* was highly induced by specific phytohormones, including cytokinin, auxin, and the defense-signaling molecule salicylic acid (SA), but not by the jasmonates. Overexpressing tomato lines were generated, and infection tests showed that, significantly, roots overexpressing *SlWRKY45* contained substantially increased number of females, indicating that *WRKY45* overexpression supported faster nematode development. Overall, this study indicated SlWRKY45 to be a potential transcription factor whose manipulation by the invading nematode might be critical for coordination of hormone signals supporting favorable condition for nematode development in root tissue.

PLANT-PARASITIC NEMATODES ON CORN (*ZEA MAYS*) AND THEIR ASSOCIATION WITH ABIOTIC FACTORS IN NORTH DAKOTA. **Chowdhury, Intiaz, G.P. Yan, and A. Plaisance.** North Dakota State University, Department of Plant Pathology, Fargo, ND 58108.

Plant-parasitic nematodes (PPN) are an important group of pathogens that affect corn production in the United States. However, extent of impact of these nematodes on yield is influenced by soil properties, climatic factors and nematode populations. In the neighboring state of South Dakota, root-lesion nematodes were reported to cause yield loses of up to 4.0 bu/ha in corn fields. Very little information exists about PPN occurrence in North Dakota corn fields. Thus nematode surveys were conducted to assess the incidence and abundance of PPN in corn fields of ND and their relationship with these abiotic factors. In 2015, samples were collected from 200 corn fields across 19 counties, and from 100 fields across 18 counties in 2016. Soil samples were taken from a depth of 15 to 30 cm below ground level and nematodes were extracted via centrifugal sugar flotation method and counted under light microscope. Correlation analyses were performed to assess the relationship between soil properties or climatic factors, and PPN densities. In 2015, 92% of the fields surveyed were positive for PPN. Eight major groups of PPN were identified including spiral (prevalence: 58%; highest density: 16,910 per kg of soil), stunt (39%; 9,500), pin (27%; 7,800), root-lesion (17%; 1,225), dagger (7%; 875), lance (3%; 380) and stubby root (1%; 200) nematodes as well as soybean cyst nematode (SCN) juveniles (11%; 4,500). In 2016, 73% of the fields surveyed were positive for PPN infestation. Eight major genera of PPN were identified and quantified including spiral (40%; 6,965), stunt (33%; 4,020), pin (40%; 6,889), root-lesion (24%; 1,905), dagger (10%; 804), lance (3%; 448) and stubby root (2%; 45) nematodes. Juveniles of SCN (8%; 1,022) were also detected. In order to determine the relationship between these nematode populations and the various edaphic factors, Pearson's correlation analysis was conducted. A significant negative correlation was found between pin nematode populations and soil pH (r: -0.4; *P*: 0.0002). A significant positive correlation existed between the percentage of sand and nematode populations of root-lesion (0.2; 0.04) and stunt nematodes (0.2; 0.02). We also observed significant negative correlations between the percentage of clay and the nematode populations of spiral (-0.2; 0.04), root-lesion (-0.3; 0.02) and stunt nematodes (-0.2; 0.02). In 2016, generally similar trends were observed, however, significant correlations were not observed. In order to investigate the influence of climatic factors on the incidence and abundance of these nematodes, meteorological data were collected from North Dakota Agricultural Weather Station. Results of correlation of PPN with weather parameters indicate that towards the end of the summer and the beginning of winter, nematode populations increase with the decreasing temperature. On the other hand, we can conclude that increased rainfall can lead to greater plant-parasitic nematode populations. With such research findings, we are better equipped to assess if plant-parasitic nematodes have a potential to significant impact on corn production in North Dakota fields.

BIOLOGICAL CONTROL APPROACHES TO PLANT-PARASITIC NEMATODES: THE CASE OF TRICHODERMA HARZIANUM STRAIN THZID1. **Contina, Jn-Bertrand, L.M. Dandurand, G.R. Knudsen**. Entomology, Plant Pathology and Nematology Dept., University of Idaho, Moscow, ID 83844-2339.

Plant-parasitic nematodes represent a threat to many economically important crops throughout the world. Between 8 to 20% of crop losses are caused by nematodes with an estimated cost of $87 billion annually worldwide. Control of plant parasitic nematodes often depends on the application of nematicides in infested fields. However, due to negative environmental impacts, many nematicides have had to undergo re-registration with the Environmental Protection Agency (EPA), leaving at risk many susceptible crops. The use of biological control agent is intended to control a plant pathogen by introducing beneficial organisms as well as to maintain, restore or enhance the natural suppressive mechanisms in soil. *Trichoderma harzianum* strain ThzID1 was isolated from Palouse silt loam soil on the University of Idaho Plant Science Farm in Moscow, ID. *Trichoderma harzianum* ThzID1 was able to control important soilborne fungal diseases as well as to reduce significantly the potato cyst nematode *Globodera pallida* reproduction rate in roots in greenhouse experiments. Transformed with green fluorescent protein, the biocontrol mechanisms used by *Trichoderma harzianum* ThzID1-M3 comprised: (1) Proliferation into the rhizosphere and rhizoplane; (2) Direct colonization of *G. pallida* juveniles and cysts; (3) Increase plant biomass; and (4) Potential induction of systemic resistance in plants. In general, biocontrol agents require a period of time to establish an economic level of pest suppression. Biocontrol activities are best enhanced through mycelia

production in oat or other substrates prior to soil application. Soil moisture, aeration, and temperature should be optimal for mycelial development. Potential fungus-feeding nematode, organic compounds or other soil microorganisms could have a detrimental effect on *T. harzianum* biocontrol activities. The addition of *Aphelenchoides* sp., a fungivorous nematode, in soil significantly reduced *T. harzianum* ThzID1 growth. The presence of glucosinolates, organic compounds produced by *Brassica napus* seed meal, inhibited *T. harzianum* ThzID1 proliferation in soil. To be successful in the long-term, biocontrol agents in general must be further studied and evaluated for understanding their interactions with the pathogen or nematode target, the crop and the soil ecosystem.

A SPATIAL POINT PATTERN ANALYSIS OF THE POTATO CYST NEMATODE GLOBODERA PALLIDA IN SOUTHERN IDAHO. **Contina, Jn-Bertrand, L.M. Dandurand, G.R. Knudsen**. Plant, Soil and Entomological Sciences Dept., University of Idaho, Moscow, ID 83844-2339.

The potato cyst nematode (PCN) *Globodera pallida* is a quarantine potato pest in the state of Idaho where it was discovered in 2006 for the first time in the U.S. PCN is characterized by the development of a globose cyst containing several hundred of eggs. PCN can survive in soil for decades and this pest is spread over long distances by contaminated soils, tubers or by contaminated agricultural machinery. A spatial point pattern analysis was used to: (1) understand the spatial arrangement of PCN infested fields in Southern Idaho; and (2) evaluate the potential threat of PCN for entry to new areas using spatial interpolation in the absence of quarantine measures. Infested fields represented as point coordinates and total cysts recovered from each field were collected by USDA-APHIS during 2006-2014. R software was used as a language environment for modeling and statistical computing. Kernel density estimation (KDE), quadrat analysis, K-function, and maximum absolute deviation (MAD) were computed using the GISTools, sp, and spatstat packages in R. Spatial interpolation was estimated using nearest neighbor (NN), inverse distance weighting (IDW) and Kriging methods, and were computed using the gstat, maptools and deldir packages in R. Results showed the presence of spatially clustered data points ($P < 0.05$), as confirmed by the quadrat and MAD tests, departing from the null hypothesis of complete spatial randomness (CSR). In the absence of quarantine measures, PCN incidence increased in interpolated areas with the presence of focal points of high PCN densities. Quarantine activities are facilitated by the presence of clustered PCN infested fields. Initial PCN field infestations seemed to begin from a focal point of high cyst densities which increased in diameter in time. To our knowledge, this is the first use of spatial analysis for understanding PCN distribution in Idaho using R. The tools and methods provided in this study should facilitate comprehensive approaches to improve PCN control and eradication programs, as well as to raise public awareness of the problematic of this economically important potato pest.

MANAGEMENT OF *MELOIDOGYNE INCOGNITA* ON *PITTOSPORUM TOBIRA* WITH FLUENSULFONE AND FLUOPYRAM. **Crow, William, T**. University of Florida Entomology and Nematology Department, PO Box 110620, Gainesville, FL 32611.

*Pittosporum tobira* is a common landscape plant and cut foliage crop in Florida. The most important soilborne disease of *P. tobira* in Florida is root-knot disease caused by *Meloidogyne incognita*. In the past, cut foliage growers relied on fenamiphos for management of *M. incognita*. However, the withdrawal of fenamiphos in the U.S. has left cut foliage growers without effective treatment options. Two new nematicides, fluensulfone and fluopyram, were recently labeled for use on other crops but are not currently labeled for use on ornamentals. A one year pilot field trial was conducted to determine if more intensive studies to support supplemental labeling of these nematicides for use on cut foliage crops is warranted. This trial was conducted on a commercial cut foliage farm growing *P. tobira* infested with *M. incognita*. The experimental design was randomized complete block with four replications of six treatments. Treatments were: *i*) Untreated control, *ii*) fluensulfone 1.5% G with 1 application of 4 kg a.i./ha , *iii*) fluensulfone 1.5% G with 2 applications of 2 kg a.i./ha, *iv*) fluensulfone 40% EC with 1 application of 4 kg a.i./ha , *v*) fluensulfone 40% EC with 2 applications of 2 kg a.i./ha, vi) fluopyram 34.5% SC with 2 applications of 500 g a.i./ha. Treatment effects on *M. incognita* were inconclusive, but yields were significantly increased by a single application of 4 kg/ha of fluensulfone EC and by two applications of 500 g/ha fluopyram SC which increased yields relative to the untreated control by 79% and 59%, respectively. These results are promising and justify additional research on rates and timings of fluensulfone and fluopyram for practical management of *M. incognita* on *P. tobira*.

STATUS OF *GLOBODERA PALLIDA* IN IDAHO—RESEARCH OUTLOOKS. **Dandurand, Louise-Marie.** 875 Perimeter Drive MS 2339, University of Idaho, Moscow, ID 83844-2339.

The economically important nematode parasite of potato, *Globodera pallida*, is limited in host range to potato and a few other solanaceous crops, and is well adapted to survive in soil for many years. A regulated pest worldwide, its discovery in Idaho in 2006 prompted phytosanitary containment and eradication measures. Because *G. pallida* poses a substantial economic risk to the entire Idaho potato industry it is regulated by both USDA-APHIS and ISDA and is the focus of intense containment and eradication efforts. As of 2017, 9,333 acres of Idaho farmland are regulated, of which 3,047 acres are infested with *G. pallida*. Stringent adherence to this program has contained *G. pallida* to two counties in Idaho which is less

than 1% of the total acreage planted to potato in Idaho. While fumigation with methyl bromide has been effective for eradication, use was discontinued in 2015. Research goals are to develop and deploy alternative eradication measures for *G. pallida*, to effectively replace the lost use of methyl bromide. A comprehensive strategy including the use of (1) the trap crop, *Solanum sisymbriifolium,* which stimulates nematode hatching without allowing development, (2) effective use of the biofumigant *Brassica juncea* extracts that kills *G. pallida* eggs; or (3) potential use of biofumigant *Sinapis alba* extracts which enhances hatch stimulation of the nematode will be discussed. To achieve the U.S. goal of eradication, and maintain *Globodera*-free deregulated potato production acreage, growers will need access to potato cultivars with complete resistance to the spectrum of species and pathotypes of *Globodera* that are currently present and/or which could potentially be introduced. Presently, there is very little potato germplasm available in the US with resistance to *G. pallida*. Initial efforts to develop resistant germplasm through the *Globodera* Alliance (GLOBAL) project will be discussed.

EFFECT OF *MELOIDOGYNE INCOGNITA* PARASITISM ON YIELD AND SUGAR CONTENT OF SUGAR BEET IN GEORGIA. **Davis, Richard F. [1], T.M. Webster[1], B.T. Scully[2], and T.B. Brenneman[3]**. [1]USDA-ARS, P.O. Box 748, Tifton, GA 31793, [2]USDA-ARS, Fort Pierce, FL 34945, [3]Dept. of Plant Pathology, University of Georgia, Tifton, GA 31793.

Sugar beet (*Beta vulgaris*) is typically grown as a summer crop for edible sugar production in the north-central and western US, but it could be incorporated as a winter crop into annual cropping systems in the southern US where the sugar would be used for biofuel and plastic production. Sugar beet roots are severely galled by *Meloidogyne incognita*, which is common throughout the southeastern US, but *M. incognita* juveniles do not penetrate roots when soil temperature is below 18 C and do not continue to develop or reproduce below 10 C. In much of the southeastern US, soil temperatures are above those levels at planting and for several months prior to harvest but below those levels for much of the day during the coldest part of the winter. We conducted a field study for two growing seasons to determine the extent to which *M. incognita* reproduced on sugar beet grown as a winter crop in Georgia and the effect of *M. incognita* on yield and sugar content. A factorial arrangement of treatments was used with four genotypes (three *M. incognita* susceptible and one resistant) planted in the fall each year in plots treated or not treated with the nematicidal fumigant 1,3-dichloropropene and harvested the following spring. Fumigation affected yield and sugar content differently in 2015 than 2016, so data were not combined for analysis. Fumigation reduced root galling and increased yield in both years, but the yield increase was greater in 2015 than 2016. The effect of fumigation on root galling was similar between years and among genotypes. Yield differed among sugar beet genotypes in both years. Fumigation increased the yield of all genotypes in 2015 but increased only the yield of one susceptible genotype in 2016; yield of the other two susceptible genotypes increased numerically but not statistically, whereas yield of the resistant genotype was numerically greater in the non-fumigated plots. In both years, fumigation affected yield of the resistant genotype less than that of the susceptible genotypes. Sugar content, measured as degrees Brix, was increased by fumigation in 2015 but not 2016, and the resistant genotype had greater sugar content than the susceptible genotypes in both years. We conclude that sugar beet grown as a winter crop in Georgia can suffer significant yield losses from RKN parasitism, which may result in reduced sugar content in some years.

EVALUATION OF NEW NON-FUMIGANT NEMATICIDES IN FLORIDA. **Desaeger, Johan**. Department of Entomology and Nematology, University of Florida, Gulf Coast Research and Education Center, Wimauma, FL 33598.

New and upcoming non-fumigant nematicides (fluensulfone, fluopyram and fluazaindolizine) were tested in tomato and strawberry trials near Wimauma, FL in fall 2016 and spring 2017. Products were applied via drip tape and evaluated for their effects on the severity of plant root damage, nematode soil and root population density, and crop vigor and yield. Root-knot nematode (*Meloidogyne javanica*) was the predominant plant-parasitic nematode in the tomato trials, and sting (*Belonolaimus longicaudatus*) and lesion (*Pratylenchus penetrans*) nematodes were the major nematodes in the strawberry trials. No negative effect was observed on plant growth with any of the new nematicides. All nematicides reduced root-knot nematode damage compared to the control in the fall tomato trial. In the strawberry trials, nematode pressure was high in one trial, and low in the other trial, and nematode control was variable. In contrast to the metam potassium fumigant standard, the new nematicides did not show any negative effect on non-plant-parasitic nematode feeding groups (bacterivores, fungivores and omnivores). Tomato plants were more vigorous and had greater yield following metam potassium as compared to the non-fumigant nematicides. The new nematicides provide a valuable new tool and may offer new opportunities and more flexibility for Florida growers to manage nematodes. The use and integration of these new products in the predominantly fumigant-based vegetable and strawberry production systems in Florida is discussed.

ORGANORUTHENIUM (II) COMPLEXES REDUCED THE OXIDATIVE STRESS AND ALLEVIATES POLYGLUTAMINE MEDIATED PROTEOTOXICITY IN *CAENORHABDITIS ELEGANS*. **Devagi[1], G., A. Mohankumar[2], G. Shanmugam[2], P. Sundararaj[2], F. Dallemer[3], P. Kalaivani[4], R. Prabhakaran[1] and S.L. Hafez[5].** [1]Department of Chemistry, Bharathiar University, Coimbatore 641 046, India, [2]Department of Zoology, Bharathiar University, Coimbatore

641 046, India, [3]Laboratoire MADIREL CNRS UMR7246, Universite of Aix-Marseille, Centre de Saint-Jerome, bat. MADIREL, 13397 Marseille cedex 20, France, [4]Department of Chemistry, Nirmala College for Women, Bharathiar University, Coimbatore 641018, India, [5]U of I Parma REC, 29603 U of I Lane, Parma, ID 83660, USA.

Discovery of drugs that can extend the lifespan and delay the onset of age related neurological diseases of human beings is the most challenging mission. Synthetic compounds like organoruthenium (II) complexes with bioactive thiosemicarbazones and novel 1,2,4-Triazolo[1,5-*a*]pyridines have demonstrated a potent role in stress tolerance and longevity extension in *C. elegans*. Four new Ruthenium (II) P-Cymene complexes (1-4) were synthesized and characterized by various analytical and spectroscopic techniques. Structure of the complexes 3-4 were confirmed by X- ray crystallography. These complexes possess higher biological activity as compared with their corresponding ligands. Supercoiled plasmid DNA cleavage study showed that the complexes have the ability to cleave the DNA without the addition of any external agents. The BSA (Bovine Serum Albumin) interaction potential of the complexes indicated a strong interaction between the fluorophore and quencher with static quenching mechanism. All complexes had an excellent antioxidant activity *in vitro* with reference to DPPH against the dietary antioxidant vitamin C, a positive control. N2 worms supplemented with 1-4 at 6 μM was found to extend the mean lifespan ($p<0.0001$) under standard laboratory conditions without any adverse effects on egg lying and progeny development. Pretreatment with 1-4 conferred the resistance to juglone (a pro-oxidant) induced oxidative stress and increased the survival of *C. elegans*. Moreover, when compared with control group, 1-4 showed *in vivo* anti-oxidant ability as it was able to prolong the lifespan of *mev-1* mutant, in which the worms showed reduced lifespan by means of overproduced ROS. DAF-16/FoxO transcription factor which involves an insulin/IGF-1 signaling pathway and resides in cytoplasm relocated to nucleus followed by the binding of a signaling molecules was further analyzed by utilizing transgenic *C. elegans* strain TJ356. Worms exposed to 3-4 at 6 μM showed nuclear localization pattern while the control and 1-2 treated worms had cytoplasmic localization. Interestingly, 6 μM concentration of 3-4 transactivated the stress response gene *sod-3* and *gst-4* expression to 89.25, 71.79 % and 90.15, 73.05 %, respectively. It has been shown that, 3-4 supplementations conferred the resistance to poly-glutamine (poly-Q) mediated ASH neuronal death and behavioral dysfunction in HA759 worms expressing poly-Q tracts especially in ASH neurons. In addition, 3-4 exerts its neuroprotective effects by significantly decreasing the poly-Q aggregate score in AM141 worms *per se*. In conclusion, these results indicated that organoruthenium (II) complexes regulate various signaling pathways which confers resistance to stress, promotes longevity and neuroprotection against poly-Q mediated proteotoxicity in *C. elegans*. Further studies are required to find out the exact mechanism of complex 3 -4 on a higher model.

ACTIVATION OF ENTOMOPATHOGENIC NEMATODE INFECTIVE JUVENILES. **Dillman, Adler R.**, **D. Lu, and D.Z. Chang.** Department of Nematology, University of California Riverside, Riverside California 92521.

Entomopathogenic nematodes (EPNs) are insect parasites that are used in biological control. The host-seeking stage of these parasites is the infective juvenile (IJ). This is the only free-living stage and is a developmentally arrested third stage larva until it enters a potential host. Upon infection, IJs undergo dramatic changes to accommodate the environmental change from living in the soil to living inside a host insect. Many skin-penetrating parasites undergo a similar transition from being free-living in the soil, to being actively parasitic inside the host. There are many changes that take place during this transition including morphological and biochemical changes. Collectively, this process is referred to as activation. We have developed a sensitive, quantitative assay to study IJ activation to study this process in different species of *Steinernema*. We found that activation occurs in a temporal manner when IJs are exposed to host tissue and that the timing of activation is species-specific. We also found that activation is heavily influenced by the species of insect host that is used. Leveraging the genome sequence of *Steinernema carpocapsae* we have used RNA-seq to study gene expression dynamics during IJ activation and we have used mass spectrometry to identify the secreted proteins when IJs are activated. Activated IJs release a mix of ~500 protein products that collectively are toxic to potential insect hosts. The excreted/secreted proteins of *S. carpocapsae* (ESPs) showed high sequence similarity to mammalian-parasitic nematodes in the genera *Strongyloides*, *Anclystoma*, *Toxocara* and others. In fractionating the ESPs we have found reduced toxicity in some fractions while other fractions are more toxic than the crude ESPs. In future experiments we plan to isolate and identify individual or groups of nematode proteins that have insecticidal activity.

MOLECULAR CHARACTERIZATION OF THE EXPANSIN GENE IN *GLOBODERA PALLIDA* AND *GLOBODERA ELLINGNTONAE*. **Duarte, Aida, R. and L.M Dandurand.** 875 Perimeter Drive MS 2339, Plant, Soil and Entomological Sciences Department, University Of Idaho, Moscow, ID 83844-2339.

Potato cyst nematodes (PCN), are serious pests of potato worldwide. *Globodera pallida* was first detected in the year 2006 in Idaho, United States, and is a quarantine pest. *Globodera ellingtonae,* a new species of cyst nematode was discovered two years later in Oregon, reproduces on potato (*Solanum tuberosum* L). *G. ellingtonae* has a biotrophic relationship with the plants, however pathogenicity remains inconclusive. Like other pathogens, PCN employ effector proteins, to create and maintain a feeding site which is their sole source of nutrition. Expansin proteins, present in the nematodes, are believed to be associated to several metabolic and parasitic functions. Similar proteins are found in the plant cell wall, with important roles in developmental processes of plant such as cell growth, emergence of root hairs, and other developmental processes where cell wall loosening occurs. In this work, the partial cDNA sequence corresponding to the expansin gene was cloned from

*G. pallida* and *G. ellingtonae*. *In situ* hybridization confirmed *Gp-exp* and *Ge-exp* transcripts in the oesophageal glands suggesting their potential secretion into the host through the stylet and thus may have a role in nematode infection. To understand the potential role in the early steps of parasitism, potato cvs. Desiree or Innovator were infected with 1000 J2s of *G. pallida* and *G. ellingtonae* and the *expan1* gene expression were studied at differential time points (24h, 2 and 7, days). The potential role of this protein in the plant-nematode interactions is not yet clear but our preliminary results suggest both nematode species can the capacity of the potato defense response system is reduced by over expression of the *expan1* gene when the potato plants is infected by either species of these nematodes. Further investigation will lead to greater understanding of the putative molecular mimicry effect by the nematode effector expansin gene in modify plant cell wall structure.

EVALUATION OF *CATENARIA ANGUILLULAE* AS A BIOLOGICAL CONTROL AGENT FOR *ROTYLENCHULUS RENIFORMIS*, *HETERODERA GLYCINES*, AND *MELOIDOGYNE INCOGNITA*. **Dyer, David, N. Xiang, K.S. Lawrence.** 209 Rouse Life Science Building, Auburn University, AL 36849.

Populations of *Rotylenchulus reniformis*, *Heterodera glycines*, and *Meloidogyne incognita* were greenhouse tested for effects of the nematophagous fungus *Catenaria anguillulae* on population density. Cultures of *Catenaria* were isolated from populations of *R. reniformis* and *H. glycines* and grown on 0.6% beef extract agar. DNA was extracted from the fungal mycelium and the ITS1 and ITS4 regions were sequenced; using BLAST the sequences showed a 95% shared identity with *C. anguillulae* (GenBank accession numbers: KY606231 and KY606232). Slurries of *C. anguillulae* with $OD_{600}$ values of 0.182, 0.272, 0.377, 0.566, and 0.754 were produced to evaluate the efficiency of *C. anguillulae* as a biological control agent of the three species of nematodes. *C. anguillulae* applied as a soil drench at planting, and compared to standard nematicide Poncho/VOTiVO (Clothianidin and *Bacillus firmus),* Velum Total (Fluopyram and Imidacloprid), Avicta (Abamectin), and Counter (Terbufos). *R. reniformis* and *M. incognita* greenhouse testing found nematode reproduction as measured by eggs per gram of root were not reduced for either nematode by *C. anguillulae* when compared to the untreated control on cotton and corn. However, an increase in cotton biomass ($P \leq 0.1$) in *R. reniformis* tests was measured for two of the *C. anguillulae* concentration when compared to Poncho/VOTiVO and Velum Total ($P \leq 0.1$). *C. anguillulae* reduced *H. glycines* cysts population density in the four highest concentrations compared to the control ($P \leq 0.1$). Populations of cysts were reduce an average of 45.6 percent in these greenhouse evaluations. Soybean plant height, root weight, shoot weight, and overall plant biomass were also improved ($P \leq 0.1$) in all of the *C. anguillulae* concentrations when compared to the Poncho/VOTiVO and Avicta ($P \leq 0.1$). This suggest that *C. anguillulae* may provide a future biological tool to aid in the management of *H. glycines* on soybeans. Further evaluations are now being conducted in microplots and field settings.

INFLUENCE OF GRAFTING AND PRUNNING OF MELOIDOGYNE INCOGNITA (NEMATODA) ASSOCIATED WITH RESISTANT AND SUSCEPTIBLE SOLANUM LYCOPERSICUM CULTIVARS: WITH SPECIAL REFERENCE TO CENTRAL ASIA. **Eshchanov, Bahodire[1], G. Bird[1], and F. Zalom[2].** [1]Department of Entomology, Michigan State University, East Lansing, Michigan, [2]Department of Entomology and Nematology, University of California, Davis, California.

In Central Asia, *Meloidogyne incognita* is a key pest of *Solanum lycopersicum* under field and greenhouse conditions. Although the Mi gene confers resistance to *M. incognita*, cultivars possessing this gene are often not suitable for local production or preferred by local consumers. Grafting can be used to obtain plants with both resistance and appropriate horticultural characteristics. In addition, pruning is used under both field and greenhouse conditions to enhance fruit productivity. The objective of this research is to determine the impact of grafting and pruning on *M. incognita* associated with the Mi gene-containing *S. lycopersicum* cv Anahu and susceptible cv Rutgers under greenhouse conditions. After 120 days, the final population density of *M. incognita* was 11-fold greater on Rutgers, compared to Anahu. Homo-grafting yielded similar results, with a 19-fold greater final population density associated with Rutgers/Rutgers compared to Anahu/Anahu. With hetero-grafting, however, the final population densities associated with Rutgers/Anahu and Anahu/Rutgers were not significantly different from each other and Anahu/Rutgers had a significantly greater population density of *M. incognita* than Anahu or homo-grafter Anahu. Light and heavy pruning significantly increased the final population density of *M. incognita*, compared to that of non-pruned plants. Measurements of biomass partitioning among fruit, stem/leaf and root tissues were determined for all treatments. It can be concluded that the horticultural practices of grafting and pruning had significant impacts on *M. incognita* reproduction and functioning of the Mi gene.

IMPACT OF SOLANUM LYCOPERSICUM GRAFTING ON THE LIFE CYCLE OF *TRIALEURODES VAPORIORUM* (INSECTA) IN THE PRESENCE AND ABSENCE OF *MELOIDOGYNE INCOGNITA* (NEMATODA): WITH SPECIAL REFERENCE TO THE MI GENE AND TYPE-D TRICHOMES. **Eshchanov, Bahodire[1], G. Bird[1], and F. Zalom[2]**,[1]Department of Entomology, Michigan State University, East Lansing, Michigan, [2]Department of Entomology and Nematology, University of California, Davis California.

Plant grafting can be used to integrate pest resistance and food quality traits for successful *Solanum lycopersicum* production under greenhouse and field conditions. The Mi gene is known to confer resistance to *Meloidogyne incognita* and cross resistance to sweet potato whitefly (*Bemisia tabaci*) and potato aphid (*Macrosiphum euphorbia*). In addition, wild

species of *S. lycopersicum* are known to possess the Mi gene and resistance to greenhouse white fly (*Trialeurodes vaporariorum*). *S. lycopersicum* cv Anahu contains the MI gene and is one of the parents of all modern cultivars possessing this gene. The objective of this research is to evaluate the impact of *S. lycopersicum* cv Anahu rootstocks on the life cycle of *T. vaporariorum* in the presence and absence of *M. incognita*. The study used homo and hetero-grafting treatments with the susceptible cv Rutgers. In addition, it involved determination of the relationship between *M. incognita* and Type-D trichome density associated with three plant leaf height levels. Anahu was not resistant to *T. vaporariorum* in the absence of *M. incognita*. The presence of *M. incognita*, however, triggered resistant to *T. vaporariorum* on non-grafted Anahu and homo-grafted Anahu. There were significantly more Type-D trichomes on hetero-grafted Rutgers compare to non-grafted Rutgers and homo-grafted Rutgers in the absence, but not in the presence of *M. incognita*. Type-D trichome density was significantly greater on the upper leaves compared to mid and low leaves in the present and absence of *M. incognita*. In addition, *M. incognita* female final population density was almost two-fold greater in the presence of the *T. vaporariorum*, than in the absence of this insect. The study is an illustration of the importance of evaluating interactions among different types of herbivores in regards to their overall host biology.

CURRENT METHODS OF COMMUNICATING WITH CLIENTELE OF ROW CROP PRODUCTION IN THE MID-SOUTH. **Faske, Travis R.** Lonoke Extension Center, University of Arkansas, Division of Agriculture, P.O. Box 357, Lonoke, AR 72086.

During the past century, Extension has used various methods to inform people of existing and new developments in agriculture. However, the model and techniques used among Extension programs vary across the United States. Extension has maintained the traditional approach in Arkansas by retaining agricultural agents in every county with an active meeting agenda from winter production meetings to spring and summer in-field IPM meetings and field day demonstrations. In this model, the Extension plant pathologist are resources for agents and produces for such meetings and field visits, while maintaining an applied research program to evaluate and demonstrate those new developments in agriculture. With the advancement of digital communication technology the options to inform people has expanded, with some options being preferred by agents, consultants, and farmers in the mid-South. This presentation will cover the challenges and benefits of some of the most common methods used by Extension to extend information on the management of diseases and nematodes of row crops to clientele in the mid-South.

MOVEMENT OF FLUOPYRAM IN SANDY SOIL TO AFFECT *MELOIDOGYNE INCOGNITA* MOTILITY. **Faske, Travis R. and K. Hurd.** Lonoke Extension Center, University of Arkansas, Division of Agriculture, P.O. Box 357, Lonoke, AR 72086.

Fluopyram is nematicidal to *Meloidogyne incognita* and is being marketed as a nematicide applied as a seed treatment and in-furrow spray in cotton and seed treatment in soybean. It has limited xylem movement, thus direct contact is important for nematode suppression; however, the effective movement of fluopyram in sandy soil is unknown. The objective of this study was to determine the movement of fluopyram from treated seed and water dilutions in sandy soil. Treatments consisted of 0.15 mg abamectin, 0.25 mg fluopyram, and 0.75 mg thiodicarb + imidacloprid treated cotton seed; 0.15 mg abamectin, 0.15 mg fluopyram, and 0.13 mg *Bacillus firmus* + clothianidin treated soybean seed; while water dilutions of 25 μg abamectin and 25 μg fluopyram were used in the third experiment. Each treatment was placed on the soil surface of a 15-cm-deep sand column and irrigated with water. The soil was removed after 24 h from three, 5-cm long segments and mixed with an equal volume of water. A portion of the supernatant was placed into a well, which contained 30-40 J2 in 500 μl of water. In a fourth experiment, the total volume of irrigation was divided in smaller units and distributed over a 30 d period, but sampled as previously described. In the cotton seed experiment, a higher percentage of immotile J2 were observed from fluopyram collected in the 0-5 and 5-10 cm segments than abamectin. No nematode immotility was observed with thiodicarb or water control. In the soybean seed experiment, a higher percentage of immotile J2 were observed from abamectin collected in the 0-5 cm segment than fluopyram. No nematode immobility was observed with the biological agent or water control. The effective movement of fluopyram from treated seed differed between cotton and soybean seed, with a higher proportion moving farther from cotton seed than abamectin, but limited to the upper 10-cm of soil. In the third experiment, a higher percentage of immotile J2 were observed in the 5-10 cm segment from the water dilution of abamectin and fluopyram compared to the water control. No nematode immotility was observed past the 10-cm depth for either nematicide. In the fourth experiment, a higher percentage of immotile J2 were observed from fluopyram in the 10-15 cm segment than abamectin. The effective movement of fluopyram from water dilutions in sandy soil within 24 h was limited to the upper 10-cm of soil, which was similar to that of abamectin, but after 30 d more fluopyram was observed past 10-cm of soil than that of abamectin. Overall, a greater proportion of fluopyram was recovered as a water dilution than from treated seed, suggesting nematicide retention on the seed coat.

BIOFORENSIC STUDIES IN *ROTYLENCHULUS RENIFORMIS* – SOURCES AND ORIGIN**. Fatdal, Lilly, M. Melzer and B. Sipes.** Department of Plant and Environmental Protection Sciences, University of Hawaii, Honolulu, HI.

The reniform nematode, *Rotylenchulus reniformis*, infects a large number of cultivated plants in tropical, subtropical, and temperate soils worldwide. The origin of the *R. reniformis* population on the island of Oahu and its dispersal pathways across the State of Hawaii is currently unknown. This information would be useful to develop effective risk mitigation tactics against other threating nematodes. Microsatellite markers (SSR) and pedigree analysis provide an understanding of the dispersal of organisms. This study aims to determine if SSR markers found in *R. reniformis* have sufficient variation and independence to determine the pedigree of different populations of *R. reniformis*. DNA was extracted from a population of *R. renifomis* from Oahu maintained in the greenhouse. Ten polymorphic SSR markers previously evaluated on populations of *R. reniformis* from the southeast United States, Colombia and Japan were tested on 348 individuals from the Oahu population. The markers were characterized as RR2-5, RR2-6, RR3-3, RR3-8, RR4-1, RR4-4, RR4-5, RR1-5 and RR2, RR5. The Oahu population displayed variation within the loci. The RR2-5 marker produced 8% double bands and 72% single bands. Similar double bands were observed in RR2-6 (8% double bands, 56% single band, and 36% no band). RR3-3 produced 100% single bands whereas RR3-8 and RR4-1 had 40% single bands and 60% no bands. RR4-4 gave 54% single bands and 46% no bands. RR1-5, RR2, RR4-5 and RR5 did not amplify any DNA in the Oahu population. These SSR markers have detected differences within the Oahu population and differences between the Oahu population and populations outside of Hawaii. The variation detected may indicate that the Oahu population is distinct compared to isolates tested by Leach et al. Additional SSR markers and nematode populations from neighbouring Hawaiian Islands will be tested to determine which markers might be linked. Markers will be used to conduct maternity analysis, paternity analysis, and parent pair analysis to help determine dispersal from island to island.

THE EFFECT OF FLUENSULFONE (NIMITZ®) ON CYSTS OF THE POTATO CYST NEMATODE *GLOBODERA PALLIDA*. **Feist, Emily[1], C. Lilley[2], P. Urwin[2], V. O'Connor[1] and L. Holden-Dye[2].** [1]Biological Sciences, Building 85, University Road, University of Southampton, Southampton SO17 1BJ, UK. [2] School of Biology, Faculty of Biological Sciences, University of Leeds, Leeds. LS2 9JT, UK.

Fluensulfone, (Nimitz®), a novel nematicide with a distinct mode of action, has a low toxicity profile toward non-target organisms and a significantly reduced environmental impact. It has previously been shown to have irreversible nematicidal effects on the model organism *C. elegans* (Kearn et al., 2014) however it is most potent and efficacious against plant parasitic nemodes, including *Meloidogyne* spp. (Oka et al., 2009, Oka et al., 2012) and the potato cyst nematode, *Globodera pallida* (Kearn et al., 2017). In this study we are investigating the effect of fluensulfone on *G. pallida* hatching. *G. pallida* includes both diapause and quiescent stages in its life cycle in the form of encysted eggs, which can survive in the soil for over 30 years. A phenomenon common to cyst nematodes is host-nematode synchronization of hatching where hatching is stimulated by the presence of host root diffusate. For *Globodera spp*. diapause is broken after an exposure to a period of cold and followed by quiescence, which is broken by exposure to host root diffusate. This activates nematode hatching, which involves changes to the eggshell, activation of the metabolically inactive juvenile and eclosion. Fluensulfone, at concentrations as low as 1μM (0.29 ppm), completely inhibits *G. pallida* hatching from cysts for a period of 28 days. This effect was reversible at concentrations ≤5μM (1.46 ppm), partially reversible at concentrations ≤50μM (14.6 ppm) and irreversible at the maximum concentration tested of 500μM (146 ppm). Cysts exposed to ≥50μM fluensulfone contained unhatched eggs that appeared granular with the structures of the enclosed J2 no longer visible, suggesting that the cyst and eggshell are not able to protect the enclosed J2 at these high concentrations. This explains why the effects of fluensulfone at these concentrations are irreversible. At the lower concentrations tested, the unhatched J2s appeared the same as controls. Cysts treated at the lower concentrations showed recovery, suggesting fluensulfone is halting the hatching process in some way. Investigating this effect further will improve understanding of the novel crop protecting action of fluensulfone.

THE ROLE OF PLANT DEFENSE PATHWAYS IN MEDIATING NEMATODE INTERACTIONS BELOWGROUND. **Filgueiras, Camila C.[1], D.S. Willett[2], L.W. Duncan[2].** [1]University of Lavras, Lavras Minas Gerais, Brazil. [2]Citrus Research and Education Center, University of Florida, 700 Experiment Station Road, Lake Alfred, FL 33850.

Nematodes rely on volatile signals to navigate dynamic environments and locate critical resources belowground. Plants produce some of the most prevalent and behaviorally active volatile signals. Mediating production of these belowground signals are plant defense pathways that integrate above and belowground stimuli while affecting signal release. Here, we examine the role of plant defense pathways in regulating tritrophic interactions between plants, herbivores, and entomopathogenic nematodes belowground. We discuss recent results investigating the link between aboveground stimulation of plant defense pathways and entomopathogenic nematode recruitment belowground while highlighting factors influencing and opportunities for enhancing biological control.

PLANT-NEMATODE INTERACTOME: A TRANSCRIPTOMIC AND METABOLOMIC INSIGHT. **Fitoussi, Nathalia[1,2], E. Borego,[3] M.V. Kolomiets[3], N. Sela[1] and S. Braun-Miyara[1].** [1]Department of Entomology, Nematology and Chemistry units; Agricultural Research Organization (ARO), the Volcani Center, Bet Dagan, 50250, Israel. [2]Department of Plant Pathology and Microbiology, the Faculty of Agriculture Food & Environment, the Hebrew University of Jerusalem, Rehovot, 76100, Israel.

Recent findings have shown that successful long-term relationship between the biotrophic sedentary endoparasitic root-knot nematodes (RKN), *Meloidogyne spp.,* and their hosts rely on a well programmed secretion of effector proteins. Previous studies has shown that effectors interfere with and mimic multiple physiological and morphological mechanisms, leading to modifications and reprogramming of the host cells' functions, thus enslaving the cells to complete their life cycle. Herein we aimed to reveal novel effectors carrying an immunosuppression activity, through oxylipins, oxygenated lipids derived from polyunsaturated fatty acids, modulation. For that purpose a metabolic profiling of fatty acid derived molecules composition in tomato hairy roots was conducted resulted in identification of oxylipin products that are specifically altered upon tomato root inoculation with the RKN, *M. javanica,* using LC-MS/MS, suggesting that different oxylipins are maneuvered by the nematode-secreted effectors. Moreover, RNAseq was performed and generated a total of 6335 DEGs of *M. javanica* second stage juveniles (J2s) exposed to tomato protoplast, 9-HOT and 13-KOD oxylipins. The main groups of genes that have shown to be differentially expressed include genes carrying a predicted secretion signal peptide, genes involved in cell wall degradation and hormone metabolism. Among the DEGs including signal peptide, several had homology with known effectors in other nematode species, other unknown potentially secreted proteins may have a role as RKN's effectors, interacting with lipid signaling. This research will provide a better understanding of oxylipins function in regulating the outcome of the parasitic interaction.

ARE EARTHWORMS A CATALYST FOR NEMATODE INFECTION ON GOLF COURSES? **Foshee, Mary, K. Lawrence, D. Held, N. Xiang, M. Hall, S. Till**, **W. Groover, and D. Dyer.** Plant Pathology Dept., Auburn University, Auburn, AL 36849.

Earthworm presence is a common occurrence on many golf courses throughout the Southeast. While worm presence is a sign of healthy soil and turf, the casting deposits earthworms leave on top of the ground's surface causes a number of issues. On a golf course and in other turf situations, we hypothesize that earthworms will be able to transmit and relocate plant parasitic nematodes across golf greens, fields, and lawns. Castings were collected on 5 golf greens as well as the practice green at Alexander City's Willow Point golf course and 4 turf plots at Auburn University's Turfgrass Research Unit. Castings were standardized to 5ccs of soil and all nematode extractions from worm castings and gut contents were completed using the sucrose centrifugation method. Data was analyzed using SAS 9.4. The castings collected so far at Auburn University's Turf grass Research Unit found species diversity within the castings and earthworms. Castings contained an average of 219 Ring (*Criconemoides*), 290 Spiral (*Helicotylenchus*), 157 Root-knot (*Meloidogyne*), and 102 Reniform (*Rotylenchulus*) nematodes per 5ccs of worm castings. Spiral made up about 38% of each sample from the Turfgrass Unit followed by Ring at 28%, Root-knot at 21%, and Reniform at 13%. Willow Point golf course worm casting sampling found Root-knot in 68% of each 5cc casting samples, followed by Spiral at 21%, and Ring at 11%. Greenhouse trials were conducted to confirm if common earthworm species, *Eisenia foetida* and *Lumbricus terrestris* often found in turf grass are capable of dispersing plant parasitic nematodes. Casting collection and extractions resulted in 56 (58%) Root-knot, 41 (42%) Reniform, and 0 Soybean cyst (*Heterodera*) nematodes per 5ccs of worm castings. Nematode extractions from worm gut contents to confirm the nematodes were ingested by the earthworms found an average of 21 Root-knot J2 and 15 Reniform vermiform life stages were present in the gut of 40 individual dissected *Eisenia foetida* and *Lumbricus terrestris*.

THREONINE HOMEOSTASIS PLAYS A ROLE IN SUCCESSFUL ROOT-KNOT NEMATODE INFECTION OF ARABIDOPSIS. **Frey, Timothy S., C.G. Taylor.** Department of Plant Pathology, Ohio State University, Ohio Agricultural Research and Development Center, Wooster, OH, 44691.

Pathogenic organisms that have a biotrophic lifestyle, such as the southern root-knot nematode (RKN, *Meloidogyne incognita*), require a living, compatible, host cell from which to obtain nutrients for their growth and development. This close connection necessitates manipulation of host metabolism and the transfer of nutrients from the host to the parasitic nematode. Small changes in host primary metabolism may lead to significant changes in the ability of nematodes to parasitize their hosts. RKN and other plant-pathogenic nematodes must obtain essential amino acids from their host plants, among these essential amino acids are the aspartate derived amino acids. The aspartate-derived pathway includes the amino acids isoleucine, lysine, methionine and threonine. In previous studies it was shown that changes in aspartate-derived pathway homeostasis can have an impact on plant immunity to biotrophic pathogens. Modifications in threonine biosynthesis and/or catabolism in the host may render plants unsuitable to RKN infection. In this study we used mutants of *Arabidopsis thaliana* threonine catabolism enzymes, threonine aldolase (*tha1*) and threonine deaminase (*omr1*), to investigate the impact of changes in threonine metabolism on RKN parasitism. Our results reveal that disturbance of threonine homeostasis leads to a decreased ability of the nematode to effectively parasitize the host plant. Furthermore, under high RKN competition levels, we observed exaggerated decreases in fecundity and increases in male production when threonine homeostasis was disrupted. This study shows that threonine homeostasis is important for the development and reproduction of RKN.

OPDA HAS KEY ROLE IN REGULATING PLANT SUSCEPTIBILITY TO THE ROOT-KNOT NEMATODE *MELOIDOGYNE HAPLA* IN ARABIDOPSIS, **Gleason, Cynthia[1,4], N. Leelarasamee[1], D. Meldau[2], and I. Feussner[2,3].** [1]Department of Plant Molecular Biology and Physiology, Georg August University, Albrecht von Haller Institute, Göttingen, Germany, 37077, [2]Department of Plant Biochemistry, Georg August University, Albrecht von Haller Institute, Göttingen, Germany 37077, [3]Department of Plant Biochemistry, Georg August University, Göttingen Center for Molecular Biosciences (GZMB), Göttingen, Germany,37077, [4]Current Address: Department of Plant Pathology, Washington State University, Pullman, WA 99164. USA.

There is a critical need to reduce the use of nematicides in nematode management. In order to develop the tools for engineering new nematode resistance in important crop plants like potatoes, we must first understand the molecular components of plant defense and the nematode strategies used to overcome these defenses. In this effort, we studied the phytohormone jasmonic acid (JA), which plays important roles in regulating plant defenses against necrotrophic pathogens and herbivorous insects, but whose role mediating the plant responses to root-knot nematodes has been unclear. We observed that exogenously applied methyl-jasmonate (MeJA) induced root-knot nematode resistance in the model plant Arabidopsis. Interestingly, MeJA-induced resistance was independent of the JA-receptor AtCOI1 (CORONATINE INSENSITIVE 1). AtCOI1 is a central player in a majority of JA-mediated defense signaling. However, there is a body of work that suggests that oxylipins other that JA are also involved in defense signaling. For example, the JA precursor *cis-(+)*-12-oxo-phytodienoic acid (OPDA) has been shown to trigger signaling in COI1-indendent manner. With this in mind, we studied the potential role of OPDA in the plant-nematode interaction. By measuring specific metabolites in MeJA-treated Arabidopsis, we found an accumulation of the JA precursors OPDA and JA/JA-Isoleucine, indicating that exogenous MeJA treatment triggers a positive feedback loop in JA biosynthesis. We also performed nematode bioassays in several Arabidopsis mutants in JA-biosynthesis, perception, and/or signaling. Plants deficient in the biosynthesis of JA and OPDA were hyper-susceptible to the root-knot nematode *Meloidogyne hapla*. Interestingly, the *opr3* mutant, which cannot convert OPDA to JA, exhibited wild-type levels of nematode galling. In fact, additional studies showed that all mutants tested in the JA-biosynthesis and perception which lie downstream of *opr3* displayed wild-type levels of galling. The data put OPR3 (OPDA reductase 3) as the branch point between hyper-susceptibility and wild-type like levels of disease. Based on the mutant data, we suggest that the JA-precursor OPDA, not JA/JA-Ile, is a key defense signaling molecule involved in regulating plant susceptibility to nematodes.

IDENTIFICATION AND CHARACTERIZATION OF *BACILLUS SUBTILIS* INDUCED DEFENSE PROTEINS USING PROTEOMIC TECHNOLOGY AGAINST THE ROOT KNOT NEMATODE. **Govindasamy, Kavitha.** Assistant Professor (Nematology) Forest College and Research Institute, Tamil Nadu Agricultural University, Mettupalayam 641 301, Tamil Nadu India.

*Bacillus subtilis* strains are important groups of natural biocontrol agents that produce a broad spectrum of bioactive peptides with great potential against phytopathogens. They produce lipopeptide antibiotics *viz*., surfactin and iturin which impart biocidal activity in direct suppression of plant-parasitic nematodes. Root knot nematode, *Meloidogyne incognita* is an economically important pathogen of agricultural and horticultural crops including noni, a herbal medicine gaining popularity in India. In this investigation, endophytic strains of *B. subtilis* were isolated from noni plants and tested for their nematicidal activity against root knot nematode, *in vitro*. The genomic DNA of the *Bacillus* strains was isolated and amplified by PCR to identify antibiotic genes *surfactin* and *iturin*. The strain Bs 5, with high surfactin and iturin showed nematicidal activity in terms of egg hatching and caused juvenile mortality. Using the 'omic' approaches effort were taken to understand pathogenicity and defence-related genes and proteins expressed during the three way interaction of host, pathogen and biocontrol agent during disease development. Protein profiling was done using (2-DE) two-dimensional polyacrylamide gel electrophoresis and the differentially expressed proteins were analyzed by mass spectrometry. Up and down regulated protein spots were excised and analyzed by MALDI-TOF MS/MS, followed by cross-species protein identification. A total of 15different proteins were found to be differentially expressed. Proteomic investigations revealed that certain functionally important defense related proteins *viz.,* Putative late blight resistance protein homolog, Toll-interleukin resistance domain containing protein, Translation Initiation factor IF1, Disease resistance protein putative Kalata-B1 and β-1,3-glucanase were induced by *B. subtilis* which are involved in the induction of defense response of host against the damaging pathogen, *M. incognita* during the host pathogen interaction. *B. subtilis* strains possessing antibiotic genes are viable candidates in the context of biological control for exploiting their potential against plant parasitic nematodes.

NITROGEN FERTILIZER RATE AFFECTS THE NEMATODE COMMUNITY IN ORGANIC AND CONVENTIONAL CARROT PRODUCTION. **Grabau, Zane[1], D.D. Treadwell[2], J.J. Perez[2], R.C. Hochmuth[3].** [1]Entomology and Nematology Department, University of Florida, Gainesville, FL 32611, [2]Horticulture Department, University of Florida, Gainesville, FL 32611, [3]Suwannee Valley Agricultural Extension Center, Live Oak, FL 32060.

Carrot production is a growing industry in Florida with about 4000 acres currently in production. Acreage is projected to increase to 10,000 acres in the next 2-3 years, about 30% of which will be organic. Florida carrot producers face challenges managing pests, such as plant-parasitic nematodes, and choosing fertilizer rates that ensure a good crop with minimal nutrient

runoff. While plant-parasitic nematodes are harmful to carrot production, free-living nematodes can be beneficial for nutrient cycling and reflect soil food web function. Therefore, plant-parasitic and free-living nematode abundances were assessed in nitrogen fertilizer rate best management practice studies. Separate studies were conducted in certified organic and conventional carrot production. Treatments were fertilizer applications at 150, 200, 250, 300, and 350 lb N/acre in both systems. The nitrogen source in the organic study was a 3-2-3, commercially-available, blended fertilizer composed primarily of poultry litter. The nitrogen source in the conventional study was ammonium nitrate. Fertilizer treatments did not affect plant-parasitic nematode abundances at the organic or conventional sites at midseason (2 months after planting) or harvest (ANOVA, $P > 0.05$). Ring and pin nematodes were the predominant nematodes at the conventional and organic sites respectively, but were not related to carrot yields. Carrot yields were not affected by fertilizer treatments (ANOVA, $P > 0.05$) and average yield was 23.6 and 30.8 tons/acre in organic and conventional production respectively. Bacterivore abundances were greater at the higher, compared to lower, fertilizer rates at midseason and harvest at the organic site and at harvest at the conventional site (Fisher's protected LSD, $P < 0.05$). At harvest, fungivore abundances were greater at the higher, compared to lower, fertilizer rates at the organic site and greater at the 250 lb N/a rate than any other fertilizer rate at the conventional site (Fisher's protected LSD, $P < 0.05$). These trends suggest fertilizer application increases enrichment opportunists, organisms near the bottom of the food web that thrive on abundant, simple resources. Numerically, nematodes abundances were much lesser at the conventional site, which was fumigated to manage soilborne pests, than the organic site. Based on the faunal profile, the conventional site was degraded (Enrichment and Structure Indices less than 50) while the organic site was enriched but disturbed (Enrichment Index greater than 50, Structure Index less than 50). In summary, fertilizer application increased populations of enrichment opportunist organisms in both organic and conventional systems and the soil ecosystem was more robust in the organic system.

NEMATICIDES AND CROP ROTATION FOR MANAGEMENT OF PLANT-PARASITIC NEMATODES IN FLORIDA COTTON. **Grabau, Zane[1] and D.L. Wright[2].** [1]Entomology and Nematology Department, University of Florida, Gainesville, FL 32611. [2]North Florida Research and Education Center, University of Florida, Quincy, FL 32351.

Cotton is grown on over 100,000 acres in Florida, but cotton yield is suppressed by plant-parasitic nematodes such as reniform nematode (*Rotylenchulus reniformis*) and southern root-knot nematode (*Meloidogyne incognita*). Nematicides and crop rotation are important tactics for managing these nematodes, especially reniform nematode, for which no commercial resistant cultivars are available. In 2016, three research trials were conducted at the North Florida Research and Education Center in Quincy, FL. Trials 1 and 2 compared the efficacy of nematicide seed treatments and in-furrow Velum Total (fluopyram and imidicloprid) nematicide application at different rates. Trial 3 tested in-furrow Velum Total efficacy in the cotton phases at a long-term research site for comparing conventional and sod-based rotations. Conventional rotation was a yearly cycle of peanut-cotton-cotton while sod-based rotation was a yearly cycle of peanut-cotton-bahiagrass-bahiagrass. Reniform nematode pressure was high in all three trials (8000 reniform nematodes/100 $cm^3$ or greater at harvest in each trial) and southern root-knot nematode was also present in Trials 1 and 2. In Trial 1, nematicides did not affect yield or reniform or southern root-knot nematode population densities at harvest ($P > 0.05$). In the Trial 2, Velum Total efficacy for managing reniform nematode varied by rate (Fisher's protected LSD, $P < 0.05$), but Velum Total did not affect cotton yield or southern root-knot nematode population densities ($P > 0.05$). In Trial 3, nematicide application did not significantly affect reniform nematode population densities or cotton yield in either rotation ($P > 0.05$). Reniform nematode population densities were lesser and yields were greater in the sod-based compared to the conventional rotation (Fisher's protected LSD, $P < 0.05$) suggesting rotation was a more effective nematode management strategy than nematicide application. Despite high nematode pressure in these trials, there was very little benefit to applying nematicides in 2016 at the given location.

IDENTIFICATION OF *MELOIDOGYNE* SPECIES AND DISTRIBUTION IN ALABAMA USING MULTIPLE TECHNIQUES. **Groover, Will and K. Lawrence.** Department of Entomology and Plant Pathology, Auburn University, AL 36849.

Species identification of *Meloidogyne spp*. (root-knot nematode, RKN) is an important tool to offer growers in the state of Alabama, because it is beneficial for planning and implementing a crop rotation. Since host range is dependent upon RKN species, knowing what species is present in an infested field allows for a year-to-year crop rotation that can help lower the nematode population density. This also allows a grower to know species levels, and determine if there is a need for resistant crop varieties if they are available. The goal of this project was to evaluate multiple species identification techniques and determine the best combination of methods for implementing a practical and efficient assay for *Meloidogyne* species identification. To do this, three different techniques were implemented and evaluated for their ability to quickly and accurately identify *Meloidogyne* species in an unknown population. The techniques used in this study were morphological measurements, differential-host test, and molecular analysis. Each of these techniques were used on multiple populations, starting with a known *Meloidogyne incognita* race 3 population currently maintained in a greenhouse. This population was previously identified via the differential-host test. Initial results showed a confirmation of species with the differential-host test and PCR amplification, but morphological measurements of juveniles did not distinguish our test population from

*M. arenaria* and *M. javanica*. With the success of species identification, statewide collection began for species identification in Alabama. Overall, an estimated 75 samples from 14 counties in Alabama were collected from grower fields for species analysis. Crops sampled during collection included cotton, soybean, corn, peanut, sweet potato, squash, pepper, kiwi, and turf. Both molecular analysis (PCR) and the differential-host test were used for species identification. Primers used for PCR include those that will identify commonly found RKN species: *Meloidogyne incognita, M. arenaria, M. javanica, M. hapla, M. chitwoodi,* and *M. enterolobii*. Of these samples, 73 were identified as *Meloidogyne incognita* (97%), and two were identified as *Meloidogyne arenaria* (3%). These species were identified through the differential-host test and PCR using primer sets IncK-14F/IncK-14R (*M. incognita*) and Far/Rar (*M. arenaria*). Sampling and identification will continue to occur as new populations are found. Overall, *M. incognita* is the most prevalent species of root-knot nematode that has been found on cropping systems in Alabama during this project.

EPPO PEST RISK ANALYSIS AS APPLIED TO NEMATODES: THE EXAMPLE OF *MELOIDOGYNE MALI*. **Grousset, Fabienne[1], G. Curto[2], E. Evlice[3], J.M. Guitian Castrillon[4], G. Karssen[5], L. Den Nijs[5], C. Magnusson[6], T. Prior[7] and W. Wesemael[8].** [1]European and Mediterranean Plant Protection Organization, 21 boulevard Richard Lenoir, 75011 Paris, France, [2]Plant Protection Service - Servizio Fitosanitario Regione Emilia-Romagna, Via di Saliceto, n.81, 40128 Bologna, Italy, [3]Ministry of Food Agriculture and Livestock, Ankara Plant Protection Central Research Institute, Plant Protection Central Research Institute, Gayret Mahallesi Fatih Sultan Mehmet Bulvarı No: 66, 06172 Yenimahalle-Ankara, Turkey, [4]Tecnologias y Servicios Agrarios, S. A. - TRAGSATEC, C/Julian Camarillo, 6a. 4-d, 28037 Madrid, Spain, [5]National Plant Protection Organization, P.O. Box 9102, 6700 HC Wageningen, Netherlands, [6]Norwegian Institute of Bioeconomy Research, Hogskoleveien 7, 1431 Aas, Norway, [7]Fera, National Agri-Food Innovation Campus, Sand Hutton, York, YO41 1LZ, UK, [8]Institute for Agricultural and Fisheries Research, Burg. Van Gansberghelaan 96, B-9820 Merelbeke, Belgium.

The European and Mediterranean Plant Protection Organization (EPPO) is a regional standard-setting organization created in 1951. One of EPPO's main priorities is to prevent the introduction of dangerous pests from other parts of the world, and to limit their spread within the region should they be introduced. Measures adopted by countries to protect their territories from these introductions should be technically justified and based on International Standard. Since the 1990s, EPPO has been involved in developing schemes for PRA and the scheme currently mostly in use is PM 5/5 *Decision-Support Scheme for an Express Pest Risk Analysis* (available on the EPPO website www.eppo.org). A system has also been established to provide early warning on emerging pests and to perform PRA at the EPPO level. Expert Working Groups are convened to conduct PRAs on specific pests. This system is presented with the example of a recent PRA prepared for *Meloidogyne mali*, a polyphagous root-knot nematode described from Japan. *M. mali* has been introduced into the EPPO region in the Netherlands and Italy. It has been mostly reported in relation to *Malus* spp. and *Morus* spp. in Japan, and *Ulmus* spp. in the Netherlands and Italy, but it has a much wider host range of trees and shrubs. Uprooted trees in the Netherlands led to concerns on the potential impact of *M. mali* in the EPPO region. An EPPO Expert Working group conducted a pest risk analysis on *M. mali* in May 2016, which was reviewed by the Panel on Phytosanitary Measures (PPM) in March 2017. The main elements of the biology of *M. mali* and the outcomes of the PRA will be presented.

BERMUDAGRASS ROOT ROT DISEASE COMPLEX ASSOCIATED WITH TWO PLANT-PARASITIC NEMATODES AND *PYTHIUM* SPP. **Gu, Mengyi[1], W.T. Crow[1], P.F. Harmon[2].** [1]Entomology & Nematology Dept. University of Florida, Gainesville, FL 32611, [2]Plant Pathology Dept. University of Florida, Gainesville, FL 32611.

Bermudagrass root rot is one of the most common bermudagrass diseases on Florida golf courses. In a previous nematicide greenhouse experiment, creeping bentgrass growing in pots inoculated with *Belonolaimus longicaudatus* easily acquired *Pythium* root rot. Plant-parasitic nematodes were isolated from most *Pythium* root rot disease samples received by UF/IFAS Plant Diagnostic Center, especially *Meloidogyne* spp. An experiment was conducted to determine if *B. longicaudatus* or *Meloidogyne graminis* increased the incidence and severity of bermudagrass root rot caused by three *Pythium* spp. (*P. aristosporum*, *P. catenulatum* and *P. middletonii*). Each of these three *Pythium* isolates was inoculated separately and in combination with *B. longicaudatus* or *M. graminis* onto bermudagrass 4-week or 5-week after grass sprigging. Treatments and uninoculated control were arranged in a completely randomized design with either four or ten replications, depending on the experiment. Eight weeks after sprigging, plants were destructively sampled to measure root necrosis, root length, *B. longicaudatus* or *M. graminis* population density, and root infection by *Pythium* spp. In this experiment, *P. aristosporum* was virulent to bermudagrass while the other two *Pythium* spp. were avirulent. Both *B. longicaudatus* and *M. graminis* increased the percent infection by the two avirulent *Pythium* spp., but not *P. aristosporum*. The percent infection of *P. aristosporum* was reduced when *B. longicaudatus* were inoculated 7 days before the *Pythium*. These results indicate that plant-parasitic nematodes increase root infection by avirulent *Pythium* spp., and that *B. longicaudatus* may induce plant resistance to virulent *P. aristosporum*. Different nematode species and different *Pythium* spp. interact with each other in different ways.

NEMATICIDAL ACTIVITY OF KOREAN NATIVE PLANTS EXTRACTS. **Ha, Jihye[1], H.I. Kang[1], D.G. Kim[2], E.S. Yun[2], N.S. Park[2] and I.S. Choi[1,2].** [1]Department of Plant Bioscience, College of Natural Resource and Life Sciences, Pusan National University, Miryang 50463, Korea. [2]Nematode Research Center, Life and Industry Convergence Research Institute, Pusan National University, Miryang 50463, Korea.

Plant-parasitic nematodes cause substantial damage to many plants. Among many control practices, there have been some studies on the control of neamtodes using plant extracts. Twelve Korean native plants extracts were selected and tested against juveniles of *Meloidogyne incognita*, *Pratylenchus* sp. and *Rhabditis* sp. *Eclipta prostrata* extract was the most effective followd by *Corylopsis coreana* and *Koelreuteria paniculata*. *E. prostrata* extract killed 83.8 - 100% of nematodes at 1,000 ppm within 48 hrs. Our result suggests that *E. prostrate* could be a good candidate for the development of an environment-friendly control practices.

DESCRIPTION OF *HIRSCHMANNIELLA* (NEMATODA: PRATYLENCHIDAE) POPULATION FROM RHIZOSPHERE SOIL OF LIMPOGRASS FROM FLORIDA. **Habteweld, Alemayehu,[1] F. Akyazi[1,3], S. Joseph[1], W.T. Crow[2], and T. Mekete[1].** [1]Nematode Systematics and Biocontrol Laboratory, Entomology and Nematology Department, University of Florida, Gainesville, FL 32611, [2]Landscape Nematology Laboratory, Entomology and Nematology Department, University of Florida, Gainesville, FL 32611, [3]Department of Plant Protection, Faculty of Agriculture, University of Ordu, 52200, Ordu, Turkey.

*Hirschmanniella* population isolated from the rhizosphere soil of limpograss in Florida is described and illustrated based on morphology and molecular characters. The population is characterized by its body length of 1722 to 1915 μm, hemispherical lip region with 6 to 7 annules, stylet length 19 to 22 μm, irregularly areolated lateral fields, oval spermatheca filled with rounded sperm, intestine not overlapping the rectum, tail 120 to 149 μm usually with ventral projection with subterminal notch. Males similar to the females except shorter body length and presence of secondary sexual characteristics, 24 to 32 μm long spicules. The population from the present study is compared with two closely related species, *H. mucronata and H. oryzae*. Compared with these two species, the new population has a longer tail with ventral projection, but can further be separated by different morphological and morphometric characteristics such as length of PIJ from anterior end, excretory pore, nerve ring position and differences in ratios *c* and *c'*. Molecular sequence analysis using the D2-D3 expansion segments of 28S and the ITS rRNA sequences showed the new population is genetically distinct. D2-D3 sequence of the new population showed 99% and 95% sequence homology with an undescribed species of *Hirschmanniella* isolated from the Colorado River in Yuma, Arizona, and *H. oryze,* respectively. ITS sequence of the new population also showed 88% sequence homology with *H. oryzae*. The phylogenetic analysis of D2-D3 and ITS region grouped the new population with *H. oryzae*. Based on morphological and molecular characterstics, we propose this population as new species.

GENE CHIP FOR GENETIC CHARACTERIZATION OF LAND RACES IN RICE RESISTANT TO *MELOIDOGYNE GRAMINICOLA*. **Hada, Alkesh [1], N. Singh[2], V. Rai[2], N.K. Singh[2] and U. Rao[1].** [1]Division of Nematology, ICAR-Indian Agricultural Research Institute, New Delhi 110 012, India. [2]ICAR-National Research Center for Plant Biotechnology, New Delhi-110 012, India.

*Oryza sativa* L. commonly known as rice is second most important staple food crop cultivated around the world and an excellent model system for studying monocotyledonous plants. The productivity of this crop can be greatly hampered by rice root-knot nematode (RRKN), *Meloidogyne graminicola* in most of the rice growing countries particularly in South East Asia. Resistance in rice against *M. graminicola* could be the most valuable in alleviating this problem and therefore, it is necessary to identify potential sources of natural resistance against RRKN. Recently, we had reported two Indian varieties of indica rice to be promising sources of resistance and still innumerable number of land races available has so far not been explored. In view of this, in the present study, about 300 land races of rice collected from different parts of India were evaluated against RRKN. About 35 accessions appeared to be highly resistant in the very stringent *in vitro* condition using pluronic gel, PF-127 for short listing the accessions for further confirmation under pot culture condition. We found that 12 accessions did not even have a single gall. In some of the accessions, there was delayed penetration with very few females and eggs after 16 days. We have developed a rice chip that incorporates 50,051 SNPs from 18,980 different genes spanning 12 rice chromosomes, including 3,710 single-copy (SC) genes conserved between wheat and rice, 14,959 SC genes unique to rice, 194 agronomically important cloned rice genes and 117 multi-copy rice genes. This chip has been used for screening the resistant land races for genetic diversity analysis and association study with the cultivated varieties of rice. These land races will be ideal donors to introgress the resistant genes into the existing various popular commercial rice varieties and also in the development of new improved varieties. Further, these genotypes will be also of immense value to understand the genetic basis of plant nematode relationships.

IMPACT OF CROP ROTATION ON NEMATODE COMMUNITIES IN IDAHO. **Hafez, Saad L.[1] and P. Sundararaj[2].** [1]U of I Parma REC, 29603 U of I Lane, Parma, ID 83660, USA; [2]Bharatiar University, Coimbatore-641046, Tamilnadu, India.

An intensive survey was conducted to find out the impact of cropping systems on the distribution of nematodes under different cropping systems in Idaho. A total of 5,311 samples were collected from different agricultural cropping systems for

three years from 2013-2015. The survey showed that there exists a wide range of nematode distributions, which were most influenced by the cropping systems used in the area. Among all nematodes, the migratory endoparasitic nematode *Pratylenchus* spp. is the predominant nematode found in almost all samples. In general, the influence of mint cropping on this nematode is greater compared to other crops. *Pratylenchus* spp. was always found in samples taken where mint was previously cropped. This is in support of our earlier studies that *Pratylenchus* is one of the principal agents responsible for increasing the severity of wilt disease along with the pathogenic fungus *Verticillium dahliae*. Alfalfa is one of the crops that influence the occurrence and distribution of the sedentary endoparasitic nematodes *Meloidogyne hapla* and *Meloidogyne chitwoodi*. Whenever potato was cropped following alfalfa, the occurrence of either one or both species of *Meloidogyne* was reported. In addition, the population of *M. hapla* was always high. This demands the grower to fumigate the field even when this nematode was detected at the minimal level. Onion is one of the crops that reduce the nematode population, especially the pathogenic nematodes *M. chitwoodi*, *M. hapla* and *Pratylenchus* spp. One of the reasons attributed to this was that onions release allello chemicals that have nematicidal effects as reported in our previous studies. Inclusion of green manure also significantly reduced the nematode population as compared to other major crops cultivated in Idaho. The ectoparasitic stunt nematode *Tylenchorhynchus* is the second most widely reported nematode of the study, after *Pratylenchus* spp. It was reported in almost all the samples and was not influenced by the cropping system. One of the reasons attributed to this may be the broad host range of this nematode since most of the common crops grown in Idaho are host to this nematode. However, the intensity and severity of damage caused by this nematode is limited when compared to all other nematodes reported in Idaho. Distribution of stubby-root nematode is highest in the eastern part of Idaho and limited to a few pockets in other Idaho regions. The occurrence and population density of stubby-root nematode were greater in fields previously planted with monocots. Cereal cyst nematode (*Heterodera avenae)* was also reported in some pockets but the infested area is increasing as compared to earlier years. During the present survey none of the samples contained Potato Cyst Nematode, *Globodera pallida*.

AN IMPROVED TECHNIQUE FOR SORTING DEVELOPMENTAL STAGES AND ASSESSING EGG VIABILITY OF *GLOBODERA PALLIDA* USING HIGH-THROUGHPUT COMPLEX OBJECT PARAMETRIC ANALYZER AND SORTER. **Hajihassani, Abolfazl[1,2], and L.M. Dandurand[1].** [1]Department of Plant, Soil, and Entomological Science, University of Idaho, Moscow, ID 83844, [2]Department of Plant Pathology, University of Georgia, Tifton, GA 31793.

The Complex Object Parametric Analyzer and Sorter (COPAS™ FP-1000) is a large particle flow cytometer designed for analyzing, sorting, and dispensing objects of varying size (40 to 700 μm in diameter) including plant seeds, fungal pellets, larval and embryo stages of insects and *Caenorhabditis elegans*. We explored the potential of using this instrument to analyze and sort various developmental stages, and egg viability of potato cyst nematode, *Globodera pallida*. Initial assays to use the COPAS for sorting and enumerating cysts failed because of the high degree of miscounted debris. An extraction protocol was therefore optimized to extract cysts from soil samples using a Fenwick Can and then further separating cysts from debris by using either acetone or sugar flotation methods. Subsequently, cysts were successfully examined and sorted by optimizing the Side Scatter and Red-Fluorescence criteria on the COPAS. Additionally, we were able to analyze and separate eggs and juveniles from samples with mixed population using both the Extinction (optical density) and Time of Flight (size) sorting parameters. In a second study, separation of viable (live) and non-viable (dead) eggs was examined following staining eggs with a fluorescence dye, SYTOX Green for 24 hours, and application of Time of Flight and Green fluorescence (Green Peak Height) on the COPAS. Data were compared with the commonly used Meldola's viability assay by which *G. pallida* eggs were stained with Meldola's Blue for one week and then examined under a microscope. The COPAS proved to be effective in assessing viability by detecting two separate gates (populations); live eggs having green fluorescence peaks $< 200$ and dead eggs with the peaks $> 200$. Additionally, the COPAS detected greater live eggs than the Meldola's viability assay. By the application of this instrument, the time required for screening and analyzing nematode populations as well as egg viability can be noticeably reduced. In addition enumerating errors due to application of present manual techniques are eliminated.

RESPONSE OF TURMERIC (*CURCUMA LONGA*) SELECTIONS TO SOUTHERN ROOT-KNOT NEMATODE (*MELOIDOGYNE INCOGNITA* RACE 3). **Hall, Meredith[1], K.S. Lawrence[1], D. Shannon[2], T. Gonzalez[2].** [1]Dept. of Entomology and Plant Pathology, Auburn University, Auburn, AL 36849, [2]Department of Crop, Soil, and Environmental Sciences, Auburn University, AL 36849.

*Curcuma longa*, commonly known as turmeric, has been a staple in Southeast Asian culture, cuisine, and medicine for thousands of years. Due to recent discoveries in the anti-inflammatory and other health-promoting properties of this plant, demand of the medicinal has increased in the United States. Turmeric is undergoing evaluation as a potential cash crop for Alabama. In the summer of 2015, *C. longa* plants grown on the campus of Auburn University, Auburn, AL, showed symptoms of *Meloidogyne* infection including stunting, chlorosis, and galling on roots. Soil tests revealed the presence of *M. incognita* throughout the turmeric plots. Turmeric selections include (origin in parentheses) *C. longa* 2 (unknown), *C. longa* 3 (Hawaii), *C. longa* 4 (unknown), *C. longa* 5 (India), *C. longa* 6 (India), *C. longa* 7 (Korea), *C. longa* 8 (South Asia), and *C. longa* 9 (Vietnam). The eight *C. longa* selections were evaluated for *M. incognita* susceptibility in both

greenhouse and microplot trials. Growth parameters included plant height, shoot fresh weight, root fresh weight, and *M. incognita* eggs per gram of root were measured at termination. In both trial settings, *C. longa* 2 and *C. longa* 4 demonstrated taller plant height than all other selections ($P \leq 0.1$), and *C. longa* 2 exhibited greater shoot fresh weight compared to all other selections ($P \leq 0.1$). In the greenhouse at 60 DAI, *M. incognita* eggs per gram of root ranged from 196-1489, with *C. longa* 4 and *C. longa* 7 supporting a lower egg density than *C. longa* 9 ($P \leq 0.1$). Reproductive factor (RF) for all selections in the greenhouse experiment exceeded 1.0 (3.9-10.4), indicating all turmeric selections are excellent hosts of *M. incognita*. Eggs extracted from roots harvested from the microplot experiment at 60 DAP ranged from 301-1505 eggs per gram of root. Because all current turmeric selections grown on Auburn University's campus are susceptible to *M. incognita* and there are no nematicides labeled for turmeric production, field trials were conducted to test the efficacy of seven nematicides for use on turmeric. Velum Total (imidacloprid + fluopyram), Counter (terbufos), Avicta (abamectin), Vydate-L (oxamyl), Movento (spirotetramat), and Majestene (*Burkholderia* sp., strain A396) were applied as soil drenches to rhizomes sown in a naturally infested field located at the Brewton Agricultural Research Unit, Brewton, AL. At 90 DAP, *M. incognita* population density ranged from 34 to 477 eggs per gram of root, and population density of *M. incognita* eggs produced in all nematicide treatments were similar to the untreated control ($P \leq 0.1$). Turmeric root and shoot weights were also not affected by the nematicides. *Meloidogyne* spp. are classified as endemic pests throughout 46 of Alabama's 67 counties; because all turmeric selections grown on the campus of Auburn University are susceptible to *M. incognita* and nematicides are not an effective method of control, *M. incognita* may prove a major pest in Alabama turmeric production. More research will be required to establish best management practices, potentially including more chemical and biological nematicides, soil amendments, and expanded variety selection.

NEMATICIDAL ACTIONS OF THE MARIGOLD EXUDATE α-TERTHIENYL: OXIDATIVE STRESS-INDUCING COMPOUND PENETRATING NEMATODE HYPODERMIS. **Hamaguchi, Takahiro and H. Koichi.** Department of Environmental Biology, College of Bioscience & Biotechnology, Chubu University. 1200 Matsumoto, Kasugai, Aichi, 487-8501 Japan.

Although conventional controls of plant parasitic nematodes (PPN) have highly depended on chemical pesticides, today's trend is to prohibit or restrict chemical products due to their adverse effects on humans and the environment. Alternative methods such as the use of antagonistic plants capable of lowering the density of PPN in soil are not widely used because they are laborious. There is an urgent need to develop new agricultural chemicals that overcome these disadvantages. We are searching for candidate compounds from antagonistic plants to develop next generation nematicides. For this study, we investigated α-terthienyl, an allelochemical derived from marigold (*Tagetes* spp.) roots that is used to suppress PPN in crop fields, for its nematicidal activity on the model organism *Caenorhabditis elegans*. Nematicidal activity of α-terthienyl against *C. elegans* wild strain N2 was found to be dose-dependent. Its lethal effect *in vitro* was observed at concentrations of 5 μM after 72 hours of treatment. Next, we examined the response of oxidative stress-related enzymes, glutathione S-transferases (GSTs), catalases (CTLs), and superoxide dismutases (SODs) following treatment of transgenic *C. elegans* with α-terthienyl. Expression of two enzymes, GST-4 and SOD-1, were induced in whole body when transgenic nematodes were treated with oxidative stress chemicals. The same two enzymes were induced in the nematode hypodermis following treatment with α-terthienyl. These results are consistent with the hypothesis that this chemical quickly permeates the nematode hypodermis and acts as an oxidative stressor. We also confirmed that even the non-feeding dauer larvae, entirely covered with tough cuticle, was effectively killed by α-terthienyl treatment. Expression of the oxidative stress-related enzymes GST and SOD are regulated by the SKN-1/WDR-23 system. We confirmed the susceptibility of nematodes to α-terthienyl was increased when expressions of GST and SOD was suppressed by SKN-1 RNAi. Furthermore, we found that knocking down of WDR-23, a suppressor of the transcription factor SKN-1, induced GST expression and conferred resistance against α-terthienyl. From these results, we concluded that α-terthienyl is an oxidative stress-inducing chemical that effectively penetrates the nematode hypodermis and exerts nematicidal activity.

EXPRESSION OF *ARABIDOPSIS* FLAVONOL BIOSYNTHESIS GENES DURING INFECTION BY THE SOUTHERN ROOT-KNOT NEMATODE. **Hamamouch, Noureddine,[1,2] Chunying Li[1], Brenda S.J. Winkel[3], and Eric L. Davis[1].** [1]North Carolina State University, Department of Plant Pathology, Box 7903, 840 Method Road, Unit 4 Bldg, Raleigh, NC 27607, USA; [2]Polydisciplinary Faculty, University Sultan Moulay Slimane, Beni-Mellal, Morocco; [3]Virginia Tech, Department of Biological Sciences, Latham 409, Blacksburg, VA 24060, USA.

Host flavonoids are differentially-regulated during parasitism of plant roots by nematodes. In this study, expression of the *Arabidopsis thaliana* flavonol-specific transcription factor *AtMYB*12, and the flavonol synthase genes *AtFLS*-1, and -5, were monitored during Arabidopsis infection by the southern root-knot nematode, *Meloidogyne incognita*. Target gene expression was monitored in whole infected root systems and in the nematode-formed galls by quantitative real-time PCR and transcriptional GUS fusion expression analysis, respectively. Quantitative PCR using whole roots of infected Arabidopsis plants showed that expression of *AtMYB12* was reduced at 9dpi and strongly down-regulated at 14dpi. *ATMYB-12p::GUS* expression lines showed that expression of *AtMYB12* was strongly down-regulated specifically within nematode-formed galls at

time points consistent with qPCR results. In addition, an Arabidopsis *Atmyb12* mutant was more susceptible to nematode compared to wild type plants, suggesting that localized down-regulation of *AtMYB12* promotes successful *M. incognita* infection of host roots. Moreover, *AtCHS* and *AtFLS*-1, target genes for the AtMYB12 transcription factor, and *AtFLS*-5 were all down-regulated in nematode infected roots and also at the nematode-formed galls, suggesting that down-regulation of flavonol biosynthesis may be necessary for nematode parasitism. This hypothesis was confirmed by T-DNA experiments, which showed that *Atmyb1* and *Atmyb5* mutant plants were more susceptible to *M. incognita* compared to wild-type plants. Taken together, this study indicates that *M. incognita* reduces the expression of the flavonol specific transcription factor, AtMYB12 and flavonol synthase genes, *AtFLS*-1 and *AtFLS*-5, to successfully parasitize Arabidopsis roots.

CHANGE IN THE NEUROMUSCULAR SYSTEM DURING *HETERODERA GLYCINES* DEVELOPMENT. **Han, Ziduan, U. Reuter-Carlson and N.E. Schroeder.** Department of Crop Sciences, University of Illinois, Urbana, IL, 61801.

The soybean cyst nematode *Heterodera glycines* is an important pathogen to the soybean production. *H. glycines* hatches as a second juvenile stage (J2). The J2s move towards to the host roots, establish the feeding site and develop to adults. Both males and females become sedentary once they start feeding. However, females lose the ability to move for the rest of their life, while males regain their mobility after the final molt. To investigate the loss and regain of mobility during development, we are examining the neuromuscular system of *H. glycines*. GABA is the most prominent inhibitory neurotransmitter and GABAergic neurons are mostly motorneurons in the model nematode *Caenorhabditis elegans*. Using antibody staining, we detected GABAergic neurons in the ventral nerve cord of J2 *H. glycines*. We also found that the application of the GABA-like chemical piperazine paralyzed the J2s of *H. glycines*. To further investigate the molecular basis of GABA, we have cloned the gene encoding the key enzyme in GABA synthesis (*hg-unc-25*). To confirm the function of *hg-unc-25,* we are attempting to rescue the *C. elegans unc-25* mutant with *hg-unc-25*. We are also using RNAi to knock down *hg-unc-25* in *H. glycines*. We will test the behavioral change in the GABA-defective *H. glycines*. Finally, to investigate the role of GABA during development, we are using RT-qPCR to examine *hg-unc-25* expression at various developmental stages. To examine changes in the muscles of *H. glycines* we are using a combination of light and electron microscopy. We stained *H. glycines* with the actin-binding stain Phalloidin and found that following infection, females retain head muscles and esophageal muscles, but lose most body wall muscles. J3 and J4 males retain actin filament that appear disorganized. Preliminary transmission electron microscopy following high-pressure freezing and freeze substitution confirms our findings by light microscopy.

INHIBIBITORY AND STIMULATORY USE OF BIOFUMIGANT EXTRACTS FOR CONTROL OF *GLOBODERA PALLIDA*. **Harder, Cole, L.M. Dandurand, and M. Morra.** Plant, Soil and Entomological Science Department, University of Idaho, 875 Perimeter Drive MS 2339, Moscow, ID 83844.

*Globodera pallida*, the pale cyst nematode (PCN), is a quarantine pest that is present in some Idaho potato fields, and efforts are underway to eradicate it. We evaluated isothiocyanate-generating mustard seed meal as a biofumigant for PCN control. Ten nematode cysts, containing 350 eggs/cyst, were placed (inside nylon bags) in soil. This soil contained meal obtained from *Brassica juncea* at rates of 0.5, 1, 1.5, and 2 tons/ac, *Sinapis alba* at a rate of 2 tons/ac, and unamended soil. The cysts were incubated for 2 weeks, then removed and crushed. Egg samples were placed in potato root diffusate solution for two weeks, at which time hatched juvenile nematodes were counted. Hatching of PCN was significantly reduced by *B. juncea* at all amendment levels. In the presence of potato root diffusate, hatching of PCN was significantly increased when exposed to *S. alba* compared to exposure to bare soil only. *S. alba* meal contains the glucosinolate sinalbin. We hypothesized that the hydrolysis products from sinalbin may be responsible for the increased *G. pallida* hatch. PCN cysts were exposed to *S. alba* meal, *B. juncea* meal, *B. juncea* detoxified meal, or sinalbin hydrolysis products for 2 weeks in soil at a rate of 0.0044g meal/g soil (2 tons/ac). Results showed an increase in hatch of *G. pallida* when exposed to *S. alba* meal or sinalbin hydrolysis products, compared to unamended soil only, but only in the presence of potato root diffusate. Direct exposure to the sinalbin hydrolysis products in the absence of the hatching stimulus from potato root diffusate was also tested, but results again showed increased hatch in treatments only after exposure to potato root diffusate. Further studies are being conducted to determine whether this is indicative of breakdown of egg wall, or induction of some other type of hatching response in *G. pallida* when paired with hatching factors.

''LIVING INSECT BOMBS'' A NOVEL APPLICATION METHOD OF ENTOMOPATHOGENIC NEMATODES. **Hazir, Selcuk[1], M. Karagoz[2], D. Shapiro-Ilan[3].** [1]Adnan Menderes University, Faculty of Arts and Sciences, Department of Biology, Aydin-TURKEY, [2]Adnan Menderes University, Faculty of Agriculture, Department of Plant Protection, Aydin-TURKEY, [3]USDA-ARS, SE Fruit and Tree Nut Research Laboratory, Byron, GA 31008, USA.

As a new application approach, we tested the efficacy of releasing live insect hosts that were pre-infected with entomopathogenic nematodes against insect pests living in cryptic habitats. We hypothesized that the pre-infected hosts could carry the next generation of emerging nematode infective juveniles to hard-to-reach target sites, and thereby facilitate enhanced control in cryptic habitats. Thus, the infected hosts act as ''living insect bombs'' against the target pest. We tested this approach using two model insect pests: a chestnut tree pest, the goat moth *Cossus cossus* (Lepidiptera: Cossidae), and a lawn

caterpillar, *Spodoptera cilium* (Lepidoptera: Noctuidae). One pest is considered hard-to-reach via standard aqueous spray (*C. cossus*) and the other is more openly exposed in the environment (*S. cilium*). *C. cossus* and *S. cilium* studies were conducted in chestnut logs and Bermudagrass arenas, respectively. The living bomb approach was compared with standard nematode application in aqueous spray and controls (without nematode application); *Steinernema carpocapsae* (Rize isolate) was used in all experiments. The percentage larval mortality of *C. cossus* was 86% in the living insect bomb treatment, whereas, all other treatments and controls exhibited less than 4% mortality. The new approach (living bomb) was equally successful as standard aqueous application for the control of *S. cilium* larvae. Both methods exhibited more than 90% mortality in the turfgrass arena. Our new approach showed an immense potential to control insect pests living in hard-to-reach cryptic habitats.

NEXT GENERATION SYSTEMATICS OF FREE-LIVING NEMATODES. **Holovachov, Oleksandr.** Department of Zoology, Swedish Museum of Natural History, Stockholm, Sweden.

One of the ways to define the phrase ''next generation'' is ''a new entity that is likely to replace a previous entity of the same kind''. In technology, ''next generation'' often describes new tool with considerable improvements of efficiency, comparing to previous version of it. Both definitions can describe advancements in three different areas of systematics of free-living nematodes: descriptive morphology, identification and phylogenetics. Microscopy is an indispensable tool for descriptive morphology of nematodes. Although many species are described using light microscope alone, scanning electron microscopy (SEM) is unavoidable for proper interpretation of surface morphology. Some of its expensive and time-consuming technical limitations (fixation with hazardous reagents, critical point drying and sputter coating) have been bypassed with the introduction of ionic liquids. Fixation with ionic liquids allows SEM observation under low or high vacuum with quality comparable to traditional preparation, but is faster and avoids the need to use dangerous chemicals and additional equipment. Another technological advancement relates both to nematode identification and phylogenetics, and is called ''Next Generation Sequencing'' (NGS). For the purpose of identification, NGS technologies provide a relatively inexpensive and fast way to obtain barcode sequences for entire nematode communities. Metabarcoding process includes several crucial steps, final of which is the identification of anonymous barcodes. Precision of barcode identification relies on the completeness of existing reference databases. However, even with partially filled reference database available for free-living nematodes, phylogeny-based identification approach can place anonymous barcodes into supraspecific taxa (genera and families) with high confidence. Such partial biodiversity assessment can still provide sufficient amount of data for certain types of ecological studies. Nematode phylogenetics is expected to benefit most from the advancements in Next Generation Sequencing. Multigene (genomic or transcriptomic) datasets generated using NGS technologies provide several orders of magnitude more phylogenetically informative characters, comparing to morphology-based and single-gene phylogenies of the past. Original sequencing requirements of large amount of input genetic material could only be met for model species, and organisms that can be cultured artificially. Current extraction kits and sequencing protocols, however, are able to generate transcriptome (expressed messenger RNAs) datasets for few or even one specimen of larger nematode species, making large scale phylotranscriptomic studies of free-living nematodes an achievable goal. It is my sincere hope that the above described methodological advancements will not only improve the quality of species description, identification and classification, but will also help revitalize systematics of free-living nematodes on a global scale.

MOLECULAR DETECTION, IDENTIFICATION AND QUANTIFICATION OF *PARATRICHODORUS ALLIUS* FROM NEMATODE INDIVIDUALS, COMMUNITIES AND SOIL DNA. **Huang, Danqiong [1], G.P. Yan [1], A. Plaisance [1], N.C. Gudmestad [1], J. Whitworth[2], K. Frost [3], C.R. Brown[4], S.L. Hafez [5], Z.A. Handoo[6] , and A.M. Skantar[6].** [1]North Dakota State University, Department of Plant Pathology, Fargo, ND 58108; [2] USDA-ARS, Aberdeen, ID 83210; [3]Oregon State University, Hermiston Agricultural Research & Extension Center, Hermiston, OR 97838; [4]USDA-ARS, Prosser, WA 99350;[5]University of Idaho, Parma Research and Extension Center, Parma, ID 83660; [6]USDA-ARS, Mycology and Nematology Genetic Diversity and Biology Laboratory, Beltsville, MD 20705.

*Paratrichodorus allius* is one of the most prevalent species of stubby root nematodes distributed in the United States. It is the vector of *Tobacco rattle virus,* cause of corky ringspot disease. To manage or predict the occurrence of this economically significant nematode species, it is important to have a rapid, sensitive and reliable method for detecting and identifying this nematode under a wide range of conditions. The traditional morphological identification of stubby root nematode species is time-consuming and requires an experienced taxonomist due to similar morphometrics among closely related species. The goal of this research was to develop molecular protocols to detect, identify, and quantify this nematode species using DNA extracted from different materials, such as nematode individuals, nematode communities, or soil samples. In 2015 and 2016, nematodes were extracted from soil using the sieving, decanting and sugar centrifugal flotation methods, and stubby root nematodes were found in 109 soil samples from North Dakota, Minnesota, Idaho, Oregon, and Washington. Morphometric measurements identified the species as *P. allius* using nematode populations isolated from four fields (three from ND and one from MN). Meanwhile, genome sequencing, including regions of 18S rRNA, D2-D3 of 28S rRNA, and internal transcribed spacer (ITS) rDNA, confirmed the species identity in these four fields and further identified *P. allius* in 24 other fields. To

avoid the cost of sequencing services, conventional species-specific PCR was developed. In addition, to be able to estimate nematode population densities, quantitative real-time PCR (qPCR) assays (TaqMan probe and SYBR green) were also developed. Primers/probe were designed from ITS1 rDNA of *P. allius*. Specificity of the primers and probe was evaluated by *in silico* analysis and confirmed by experimental qPCR tests with specific amplification using DNA of target species and non-target nematode species. The conventional PCR and SYBR green qPCR detected and identified *P. allius* in DNA extracts of stubby root nematode individuals isolated from 35 soil samples and of nematode communities with mixed populations of nematodes isolated from 11 soil samples. In the qPCR assays for *P. allius* quantification, standard curves were generated using a serial dilution of soil DNA extracts from autoclaved soil harboring 10 *P. allius* individuals. The qPCR assay accurately quantified *P. allius* densities from DNA extracts of artificially infested soil, revealed by the correlation (r) of greater than 0.93 between the numbers of target nematodes quantified by two qPCR assays and added to the autoclaved soil. Using 17 natural field soil samples, the SYBR green qPCR assay (r = 0.93) performed better than the TaqMan probe qPCR assay (r = 0.89), indicated by a higher correlation coefficient between the qPCR quantified numbers and microscopically estimated numbers. Moreover, the numbers of target nematodes estimated by the SYBR Green qPCR assay were closer to the actual numbers estimated by microscopic methods. Results of this study suggest it is feasible to use molecular diagnostic procedures for *P. allius* detection, identification, and quantification.

MOLECULAR CHARACTERIZATION OF A NOVEL *HETERODERA* POPULATION IN THE CENTRAL VALLEY OF COSTA RICA. **Humphreys-Pereira, Danny A, L. Núñez-Rodríguez, L. Flores-Chaves.** Laboratory of Nematology-CIPROC, University of Costa Rica, San Pedro, Costa Rica, 2060.

Cyst-forming nematodes have not been studied in detail in Costa Rica, except for *Globodera pallida* which is widely distributed in the main potato-growing areas of the country. There is little knowledge on cyst-forming nematode inter- and intraspecific variability, host range and distribution. In a recent study related to plant parasitic nematodes associated with weeds in potato fields from the Central Valley of Costa Rica, a *Heterodera* sp. population was found in roots of *Rumex* sp. The *Heterodera* sp. population was characterized using the nuclear marker *ITS1-5.8S-ITS2* (728 bp) and a small portion of the mitochondrial marker *cox1* (431bp). The alignment of the *Heterodera* sp. *ITS* sequence generated from this study with sequences from other *Heterodera* species retrieved from GenBank showed sequence divergence levels ranging from 0.1% (1 nt) to 1.4% (10 nt) compared to *H. schachtii*, from 0.1% (1 nt) to 1.0% (7 nt) compared to *H. trifolii* and 0.3% (2 nt) to 0.4% (3 nt) compared to *H. daverti*. Sequence divergence based on the partial *cox1* gene was higher than the *ITS*, with 2.1% (8 nt) compared with *H. daverti*, 3.3-3.6% (13-14 nt) with *H. trifolii* and 8.7% (34 nt) compared to *H. schachtii*. The best-fit model of nucleotide substitution was selected based on the Bayesian information criterion (BIC) and phylogenetic relationships between the *Heterodera* sp. extracted from *Rumex* sp. and other *Heterodera* spp. were estimated using Bayesian Inference analyses. Bayesian analysis based on the *ITS* region placed *Heterodera* sp. within the *schachtii* group, with a high support value (PP=100). The phylogenetic analysis based on the *cox1* gene showed that *Heterodera* sp. was in a clade with sequences of *H. daverti* (PP=93). However, *H. daverti* formed a subclade (PP=98). The analysis of the mitochondrial marker might indicate the presence of cryptic species. Currently, this *Heterodera* sp. population is being described using morphology and additional molecular markers.

LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (LAMP) FOR DETECTION OF THE RED RING NEMATODE, *BURSAPHELENCHUS COCOPHILUS*. **Ide, Tatsuya[1,2], N. Kanzaki[1,3], P.P. Parra Giraldo[4,5], and R.M. Giblin-Davis[6].** [1]Forestry and Forest Products Research Institute, 1 Matsunosato, Tsukuba, Ibaraki 305-8687 Japan, [2]National Museum of Nature and Science, 4-1-1, Amakubo, Tsukuba, Ibaraki 305-0005, Japan, [3]Kansai Research Center, FFPRI, 68 Nagaikyutaroh, Momoyama, Fushimi, Kyoto, 612-0855, Japan, [4]Tropical Fruits Program, International Center for Tropical Agriculture (CIAT), Km 17, Recta Cali-Palmira Apartado Aéreo 6713, Cali, Colombia, [5]Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN 47907, USA, [6]Fort Lauderdale Research and Education Center, Department of Entomology and Nematology, University of Florida/IFAS, 3205 College Avenue, Davie, FL 33314-7799, USA.

As a first step in developing a quick, accurate and simple method for the diagnosis of red ring disease, the loop-mediated isothermal amplification (LAMP)-based identification procedure was applied to the causative agent, *Bursaphelenchus cocophilus* (Parasitaphelenchinae: Aphelenchoididae). Two LAMP primer sets were designed using two loci of ribosomal RNA genes, i.e., D2-D3 expansion segments of the large subunit (D2-D3 LSU), and internal transcribed spacers (ITS). Within those two sets of primers, the D2-D3 LSU primer set successfully yielded amplicons from *B. cocophilus* nematode lysate prepared from 3-year-old DESS-preserved specimens. The specificity of the primers was examined using 18 species of confamilial Aphelenchoididae nematodes and primer sensitivity was tested using a diluted series of *B. cocophilus* lysate. The primer set did not amplify the DNA from other aphelenchoidids, and sensitivity achieved by ''1/100 diluted'' *B. cocophilus* DNA (roughly 1/1500 of total DNA from a single third-stage juvenile). Because many commercially and horticulturally cultivated palm species, e.g., coconut palm, *Cocos nucifera*, African oil palm, *Elaeis guineensis*, and Canary Island date palm, *Phoenix canariensis* are susceptible to red ring disease and symptoms can be highly variable with different species and cultivars under different environmental conditions, the early and accurate detection of infected palm hosts is important for

reducing its spread. Also, a method for testing whether trap-captured American palm weevils, *Rhynchophorus palmarum*, at ports of entry or in border traps are vectoring red ring nematodes is needed. Although the specificity and sensitivity of the LAMP primer set was confirmed, more studies will be needed before practical application of the methodology. For example, the direct detection of nematodes from living (symptomless) trees and nematode-harboring weevils will need to be verified in field studies.

FIRST REPORT OF A PLANT PATHOGENS *DITYLENCHUS DIPSACI* ISOLATED FROM DIEBACK OF *PHLOX SUBULATA* IN JAPAN. **Ikuyo, Yoriko[1], I. Hideaki[2], O. Sota[1], and H. Koichi[1].** [1]Department of Environmental Biology, College of Bioscience & Biotechnology, Chubu University. 1200 Matsumoto, Kasugai, Aichi, 487-8501 Japan, [2]Ishiguro Botanical Garden, Ishiyakushi, suzuka, 513-0012 Japan.

Moss phlox *Phlox subulata* is an ornamental plant native to North America that is widely cultivated in many countries. In Japan, this plant is named ''Shiba zakura'' (turf cherry blossom) because of its leaf shape, growing and blooming style. Moss phlox is a perennial ground cover that blooms densely and simultaneously in early spring. This plant is very popular in home gardens as well as an important tourism resource. In recent years, the symptom of dieback was observed in *P. subulata* planted in the gardens of many parks in the Chubu area of Japan. *P. subulata* seedlings are widely exported for planting in many area of Japan without appropriate management. There is an urgent need to identify pathogens associated with dieback in moss phlox. We attempted to isolate nematodes from the diseased stems of *P. subulata* collected from a park in the Aichi prefecture of Japan. Based on morphological observation and molecular analysis based on the 28S rRNA D2/D3 sequence, we identified the major nematode isolated from the diseased stem was *Ditylenchus dipsaci*. *D. dipsaci* (stem and bulb nematode) is a polyphagous plant parasitic nematode distributed worldwide particularly in temperate areas. It is listed as a quarantine pest in many countries. The pathogenic and physiological traits associated with *D. dipsaci* isolates from different hosts and countries vary widely. We analyzed the phylogenetic relationship of our nematode *D. dipsaci* isolate, KHA801, with reported isolates based on the partial sequences of ITS1-5.8S-ITS2. Although many nematode isolates from a wide variety of plant hosts including one from moss phlox, have been genotyped no correlation between isolate haplotypes and host was seen by phylogenetic analysis. To confirm the pathogenicity of *D. dipsaci* KHA801 we cultured it on carrot discs and used for inoculation experiments. Three *P. subulata* seedlings were planted in a planter. The middle seedling was inoculated with about 200 of nematodes, then cultured in the greenhouse. We observed the symptom of dieback at the nematode-inoculated site, and re-isolated many *D. dipsaci* from the diseased stem. No dieback was detected on the other seedlings. From our experiments, we conclude one pathogen capable of causing Phlox dieback in Japan is *D. dipsaci*.

EVALUATION OF OIL RADISH AND WHITE MUSTARD CULTIVARS AS TRAP CROP FOR MANAGING SUGAR BEET CYST NEMATODE. **Jae-Kook, Lee[1], S.J. Kim[2], and H.R. Ko[1]. [1]** Crop protection Division, National Institute of Agricultural Science, RDA, Jeonju, 560-500, Republic of Korea, [2]2mbio co., ltd, Suwon 16229, Republic of Korea.

Cultivars of oil radish and white mustard were examined for evaluation of trap crop for managing sugar beet cyst nematode (*Heterodera schachtii*) at highland chinese cabbage field in Korea. Oil radish (*Raphanus sativus* cv. Adios, Anaconda, Bokito, Doublet, Final, Terranova) and white mustard (*Sinapis alba* cv. Architect, Attack, Braco, Vitaro) were planted in a field plot infested with *H. schachtii*. Nematode population densities were determined at planting, 2 months after planting, and 20 days after incorporation of plant material into the soil(biofumigation). The oil radish was reduced more nematode population than white mustard after biofumigation, and five oil radish(cv Adios, Anaconda, Bokito, Doublet, Final) were reduced eggs and 2nd juveniles more than 90% compared with the initial population. Approximately 50 days after susceptible Chinese cabbage (Chukwang) transplanting to biofumigated soil, nematode population and plant weights were assessed. The results from this study, oil radish cultivars Adios and Anaconda could be effective trap crop for management of sugar beet cyst nematode in Chinese cabbage field.

OCCURRENCE AND DISTRIBUTION OF PLANT- PARASITIC NEMATODES IN TURFGRASS IN GEORGIA GOLF COURSES. **Jagdale[1], Ganpati B., L.C. Arnold- Smith[1] and A.D. Martinez-Espinoza[2]. [1]**Department of Plant Pathology, University of Georgia, Athens, GA, 30602, **[2]**Department of Plant Pathology, University of Georgia, Griffin CAES Campus, Griffin, GA 30223.

Currently turfgrasses are grown in Georgia on over 1.9 million acres on landscapes, athletic fields, cemeteries, commercial buildings, golf courses, home lawns, schools and parks. Plant- parasitic nematodes (PPNs) can impact turf health, quality, and maintenance of both warm and cool season turfgrasses on golf courses. PPNs can cause an estimated 2.5% to 4.5% of reduction in turfgrass value, which translates into $54 to $72 million annually in Georgia. We examined the occurrence and distribution of PPNs on different turfgrasses in several Georgia golf courses during 2014, 2015 and 2016. At the UGA Nematode Diagnostic Laboratory, we received 1143 soil samples from 101 different golf courses located in 54 different counties that spread throughout 4 geographical regions (Coastal plain, Piedmont, Blue Ridge, and Ridge and valley) of Georgia in 2014, 2015 and 2016. PPNs extracted from soil were identified to genus level and their population densities were recorded. The major PPN genera found associated with Georgia golf course turfgrasses included *Belonolaimus* (sting),

*Helicotylenchus* (spiral), *Hemicycliophora* (sheath), *Heterodera* (cyst), *Hoplolaimus* (lance), *Meloidogyne* (root-knot), *Mesocriconema* (ring), *Paratrichodorus* (stubby), *Pratylenchus* (lesion), *Tylenchorhynchus* (stunt) and *Xiphenema* (dagger). Of these PPNs, the most frequently occurring genus in Georgia golf courses was *Mesocriconema* found in 99% of soil samples whereas the genus *Heterodera* was found only in 2% of soil samples. Distribution of PPNs varied between geographical regions of Georgia. For example, the most damaging nematodes including *Meloidogyne* and *Belonolaimus* were associated with turfgrass species in golf courses from the Ridge and Valley but not from the Blue Ridge region. Also, only *Mesocriconema, Helicotylenchus, Paratrichodorus and Tylenchorhynchus* were most abundant in Ridge and Valley than in Blue Ridge region. When comparisons were made between Piedmont and Coastal Plain regions, we found that *Belonolaimus*, *Helicotylenchus*, *Hoplolaimus*, *Meloidogyne*, *Mesocriconema, Paratrichodorus* and *Tylenchorhynchus* were frequently occurring and the most abundant genera in both the regions, but *Pratylenchus* and *Hemicycliophora* genera were abundant only in Piedmont. Furthermore, frequency of occurrence of PPNs varied by turfgrass species grown in golf courses. For example, the frequency of occurrence of *Mesocriconema, Helicotylenchus, Tylenchorhynchus* and *Paratrichodorus* was comparatively higher in bentgrass (58.79, 50.31, 46.37 and 44.00%, respectively) than in bermudagrass (39.98, 18.29, 3.85 and 14.35%, respectively). In contrast, the frequency of occurrence of *Belonolaimus* was higher in bermudagrass (23.97%) than in bentgrass (16.36%). Frequencies of occurrence of *Meloidogyne*, *Hoplolaimus* and *Hemicycliophora* were comparable in both bentgrass (26.25, 11.37 and 9.97%, respectively) and bermudagrass (21.0, 11.98 and 11.64%, respectively). Other PPNs including *Pratylenchus*, *Xiphenema* and *Heterodera* were detected in low frequencies (< 4%) in both grasses. Knowledge of PPN's species and their distribution can help golf course superintendents with developing control strategies that are consistent, promote long-term control, reduce nematicide applications, and improve turfgrass health and quality.

NEMATOTOXICITY OF VETIVER EXTRACTS AND ROOT OIL AGAINST *MELOIDOGYNE INCOGNITA*. **Jindapunnapat, Kansiree[1,2,3], S.L.F. Meyer[2], N.D. Reetz[2], M.H. MacDonald[2], G. Bhagavathy[4], B. Chinnasri[1], N. Soonthornchareonnon[5], A. Sasnarukkit[1], K.R. Chauhan[4], D.J. Chitwood[2].** [1]Department of Plant Pathology, Kasetsart University, Bangkok, Thailand; [2]USDA-ARS Mycology and Nematology Genetic Diversity and Biology Laboratory, Beltsville Agricultural Research Center (BARC), Beltsville, MD; [3]Center for Advanced Studies for Agriculture and Food, Kasetsart University, Bangkok, Thailand; [4]USDA-ARS Invasive Insect Biocontrol and Behavior Laboratory, BARC, Beltsville, MD; [5]Department of Phamacognosy, Mahidol University, Bangkok, Thailand.

Vetiver (*Vetiveria zizanioides*) produces a strong fibrous root system and dense aboveground biomass. This grass is planted for numerous reasons, including land stabilization, water conservation, bioremediation, and production of essential oil from roots. The vetiver root oil, and compounds derived from vetiver oil and vetiver plant extracts, are active against various organisms. However, very few studies have been published on effects of vetiver oil or extracts on root-knot nematodes. Consequently, vetiver oil and vetiver root and shoot extracts were tested for activity *in vitro* against the root-knot nematode *Meloidogyne incognita*. When second-stage juveniles (J2) were immersed in crude root and shoot aqueous extracts, crude ethanol root extracts, and vetiver oil, root and shoot extracts were active, killing ca. 40% to 72% of the J2. Vetiver oil was not nematotoxic. Chemotaxis assays on water agar determined that aqueous extracts from vetiver roots and shoots, and ethanol extracts from roots, were repellent to J2. Conversely, vetiver oil did not attract or repel the J2. Gas chromatography/mass spectrometry (GC/MS) analysis determined that the tested vetiver oil and ethanol extracts from vetiver roots had two major constituents in common: 3,3,8,8-tetramethyltricyclo[5.1.0.0(2,4)]oct-5-ene-5-propanoic acid (a sesquiterpene derivative), and the sesquiterpene alcohol 6-isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydronaphthalen-2-ol. The percentage areas under the GC/MS peaks indicated that the ratio of the acid to the alcohol was high in the extracts (1.4 to 8.7) and low in the vetiver oil (0.04). This difference in plant chemistry may have played a role in the nematode-antagonistic activity of the extracts vs. the vetiver oil, although the extracts and oil also contain many minor constituents. The nematotoxicity of vetiver plant extracts to *M. incognita* suggests that vetiver grass, when applied as a mulch or amendment to agricultural fields, may contribute to suppression of root-knot nematodes.

DEVELOPMENT OF MICROBIAL SOURCED NEMATICIDES FOR APPLICATION IN-FURROW AND AS SEED TREATMENTS FOR MANAGING NEMATODES IN MAJOR ROW CROPS. **Johnson, Timothy, P. Pathak, and P. Marrone**. Marrone Bio Innovations, 1540 Drew Avenue, Davis, CA 95618.

Various preparations of *Chromobacterium subtsugae* strain PRAA4-T[1] and heat-killed *Burkholderia sp*. strain A396 were evaluated from 2014-2016 as in-furrow and seed treatments for reducing damage from nematodes feeding on cotton, corn and soybeans. In-furrow studies included multiple rates applied either directly into the open seed furrow or applied as a T-band over the open seed furrow prior to the closing wheels. Trial locations were in Nebraska, Iowa, Minnesota, Wisconsin, Tennessee and North Carolina. A commercial formulation of *Burkholderia sp*. strain A396 applied at 14 and 28 fluid ounces per 1000 row-feet successfully reduced soybean cyst (*Heterodera glycine*) nematode numbers and increased grain yield in soybeans when compared to no treatment. The same treatments reduced numbers of southern root knot nematode (*Meloidogyne incognita*) in cotton and resulted in increased yields of seed cotton. Similar applications in corn also showed promise for reduction of plant parasitic nematodes (root lesion, dagger and lance) and protecting grain yield. A separate effort to

develop seed treatments based on preparations of *C. subtsugae* strain PRAA4-T1 and heat-killed *Burkholderia* sp. strain A396 on corn and soybeans yielded promising results when compared to commercial standards. Seed treatments included the nematicide component alone and the addition of various components for managing pest insects, fertility and soil-borne diseases. In addition to evaluating seed treatments for managing plant parasitic nematodes, similar studies were conducted for management of western corn rootworm larvae (Diabrotica virgifera virgifera) and seed corn maggot (*Delia platura*). Trial locations were in Nebraska, Iowa, Minnesota, Wisconsin, Tennessee and North Carolina. A comprehensive review of trial data indicates a realistic goal of developing a biological stacked seed treatment that provides a comprehensive package for managing major nematode, insect and disease pests of large acreage crops in North America.

MINIMIZING SAMPLING NUMBER FOR EXTENSIVE FIELD SURVEY IN SUGARBEET CYST NEMATODE INFESTED CHINESE CABBAGE FIELD BY USING SADIE (SPATIAL ANALYSIS BY DISTANCE INDICES). **Kabir, Faisal M. [1], J.-J. Park[2], A.O. Mwamula[1], M.-G. Jeong[1], H.-G. Kim[1], J.-G. Lee[3] and D.-W. Lee[1].** [1]Department of Ecological Science, Kyungpook National University, Sangju, Gyeongsangbuk-do 37224, Korea. [2]Department of Plant Medicine, Inst. of Agric. & Life Sci. Gyeongsang National University, Jinju, Gyeongsangnam-do 52828, Korea. [3]Crop Protection Division, National Academy of Agricultural Science, RDA, Wanju, Jeollabuk-do 55365, Korea.

Extensive field surveys are an important part of nematology to determine the occurrence, distribution, frequency, and the abundance of nematodes species over large geographic areas. However, the traditional sampling method is a time consuming and lengthy process because of its large number of sampling. Using geostatistics, variogram model analysis, could minimize the number of sampling as it can interpret the spatial structure and predict the directional anisotropy of data. Data from two highly infested sugarbeet cyst nematode in Chinese cabbage field (Jungsun and Sumcheok) had been sampled traditionally (each sample was taken apart from 2 and 5m distances), however, after analyzing the variogram modeling, the Gaussian model predicted the rising directional (independent) data range and scattered directional (dependent) data range. Using directional data we can minimize the number of samples taken by up to more than 50% compared to traditional method.

I JUST WANT TO BE VERIFIED: TWITTER SUCCESS STORIES. **Kaminski**, **J.E.** The Pennsylvania State University, 21 Tyson Building, University Park, PA 16802. kaminski@psu.edu.

Twitter has emerged as one of the premiere social media resources for extension educators. The 140 character microblog mobile app allows users to crowd source information in real time and has connected experts with an audience in the millions. Beginning in 2009, the social network was used to increase the distribution of outreach, teaching and research materials for the Penn State turfgrass programs. The @PSUTurf Twitter account currently has over 6700 followers and a monthly reach of over 20,000 impressions. The program's Twitter account is used to fulfill all three missions of the Land Grant University. In 2017, the @PSUTurf Twitter account reached ''verified'' status. The successes and failures of using the microblog platform will be discussed in detail.

DISTRIBUTION OF NEW SOYBEAN CYST NEMATODE, *HETERODERA SOJAE*, IN KOREA. **Kang, Heonil[1], J.H. Ha[1], J.H. Lee[1], E.S. Yun[2], D.G. Kim[2], N.S. Park[2] and I.S. Choi[1, 2].** [1]Department of Plant Bioscience, College of Natural Resource and Life Sciences, Pusan National University, Miryang 50463, Korea. [2]Nematode Research Center, Life and Industry Convergence Research Institute, Pusan National University, Miryang 50463, Korea.

A new soybean cyst nematode, *Heterodera sojae* was reported from Korea in 2016. Cysts of *H. sojae* appeared more round, shining and darker than that of *H. glycines*. In 2016, 270 soil samples were collected from soybean fields and examined the existence of *H. glycines* and *H. sojae*. Total of 111 samples (41.1%) contained cysts (41.1%). Among them 77% (85 samples) were *H. glycines* and 23% (26 samples) were *H. sojae*. *H. sojae* is widely distributed in soybean fields in Korea and above ground symptoms are similar to *H. glycines*. Damage potential, resistance, and ecological studies will be conducted.

NEMATODES ASSOCIATED WITH PALM AND SUGARCANE WEEVILS IN SOUTH FLORIDA WITH NOTES ON A NEW SPECIES OF *ACROSTICHUS*. **Kanzaki, Natsumi[1,2,3], R.M. Giblin-Davis[3], R. Gonzalez[3], M. Manzoor[4].** [1]Forestry and Forest Products Research Institute, 1 Matsunosato, Tsukuba, Ibaraki 305-8687 Japan, [2]Kansai Research Center, FFPRI, 68 Nagaikyutaroh, Momoyama, Fushimi, Kyoto, 612-0855, Japan, [3]Fort Lauderdale Research and Education Center, Department of Entomology and Nematology, University of Florida/IFAS, 3205 College Avenue, Davie, FL 33314-7799, USA, [4]Department of Entomology, University of Agriculture, Faisalabad, 38000 Pakistan.

During a 2016 survey of the nematode associates of the native palmetto weevil, *Rhynchophorus cruentatus*, and the recently introduced West Indian sugarcane weevil, *Metamasius hemipterus* (Coleoptera: Curculionidae) from southern Florida, an undescribed species of *Acrostichus* was cultured from a single dissected *R. cruentatus* from Fort Pierce, FL. Morphological and molecular studies showed that it was new to science. The new species is characterised by its male tail characters, spicule morphology with rounded manubrium separated from other parts by clear constriction, smoothly ventrally curved blade, slightly dorsally recurved and pointed tip, more or less straight gubernaculum with widely rounded anterior end

and a triangular (arrowhead-like) appendage at the distal tip, and the arrangement of male genital papillae, <(v1, v2), v3 / v4, ad, ph, (v5, v6, v7, pd)>. In addition to this new species of *Acrostichus* and the previously described nematode associates of *R. cruentatus*, i.e., *A. rhynchophori*, *Teratatorhabditis palmarum* and *Mononchoides* sp., we recovered a putative new species of *Demaniella* and a new association record with *Rhabditidoides humicolus* and *Diplogastrellus metamasius* in Homestead, FL. Dissections and subsequent culturing attempts with *M. hemipterus* revealed the previously described nematode associates of *Caenorhabditis angaria* and *D. metamasius* as well as a new association with *R. humicolus* in Homestead, FL.

MICROPLOT RESEARCH SUPPORTS THE EXISTENCE OF VIRULENCE PHENOTYPES IN POPULATIONS OF RENIFORM NEMATODE ENDEMIC IN LOUISIANA. **Khanal, Churamani, E.C. McGawley, C. Overstreet, and M. Kularathna.** Louisiana State University AgCenter, Department of Plant Pathology and Crop Physiology, Baton Rouge, LA 70803.

Microplot studies were conducted to assess virulence phenotypes of populations of reniform nematode (*Rotylenchulus reniformis*) endemic in Louisiana. Reniform nematode populations were derived from single egg mass collected from West Carrol, Rapides, Morehouse, and Tensas parishes in Louisiana. Trials were conducted using the upland cotton cultivars (*Gossypium hirsutum*) Phytogen 499 WRF, Deltapine 1133 B2RF, and Phytogen 333 WRF that are recommended for use in Louisiana. Experiments were established as a 3 X 4 X 5 design (cotton cultivars X isolates of reniform nematode X replications) with inoculum level of 50,000 vermiform life stages of nematodes. After 165 days, nematodes were extracted and enumerated from a 250 cc subsample of soil from each microplot. Plant data collected include number of bolls, seed cotton weight, lint weight, and plant dry weight. A significant difference in reproduction among the reniform nematode isolates was observed. Of the four isolates and across all varieties, the Morehouse isolate displayed the greatest level of reproduction; Tensas and West Carroll were intermediate; and Rapides had the least. Reproduction of Morehouse isolate was 31% greater than that of Rapides isolate. A significant reduction in plant dry weight, numbers of bolls, seed cotton weight, and lint percentage were observed for the nematode inoculated plants when compared with uninoculated controls. Plant dry weight losses caused by West Carrol, Rapides, Morehouse, and Tensas isolate was 26%, 9%, 55%, and 21%, respectively. Number of bolls, seed cotton weight, lint weight, and lint percentage reduction by the most damaging Morehouse isolate were 66%, 59%, 65%, and 14%, respectively. Similarly, number of bolls, seed cotton weight, lint weight, and lint percentage reduction by the least damaging Rapides isolate were 14%, 15%, 19%, and 4%, respectively. Results suggest that endemic populations of reniform nematode behave differently on cotton indicating virulence phenotypes. Cotton varieties, across all isolates, did not differ significantly in terms of reproduction of reniform nematodes. Nematode reproduction in DP1133 was approximately 3% higher than in PHY333, and PHY499. Data obtained from this research supports previous reports of the existence of virulence phenotypes of reniform nematode.

BREEDING WALNUT ROOTSTOCK GENOTYPES FOR RESISTANCE TO KEY SOIL BORNE PESTS AND PATHOGENS **Kluepfel, Daniel[1], A. Westphal[2], C. Leslie[3], G. Browne[1], M. Aradhya[1], J. Hasey[4], M.-C. Luo[3], and J. Dvorak[3].** [1]USDA-ARS, Crops Pathology and Genetics Research Unit Davis, CA, [2]Department of Nematology, University of California Riverside, [3]Department of Plant Sciences, University of California Davis, [4]Cooperative Extension University of California Agriculture and Natural Resources, Yuba City, CA.

In North America, 99% of the English walnuts are produced on trees which have been grafted or budded onto rootstocks which are genetically distinct from the scion. The most commonly used rootstock (~90%) is a hybrid seedling rootstock known as ''Paradox''. Paradox originates from open pollinated seeds from a cross between Northern California Black walnut (*J. hindsii*) and English walnut (*J. regia*). These genetically narrow rootstock genotypes have performed well but they offer limited protection against crown gall, Phytophthora crown/root rot and root lesion nematodes. Because there are limited post-plant remedies for these soil-borne pathogens, our goal is the development of disease resistant rootstocks and identification of the walnut genes which control this resistance. Towards that end, we exploited the wild species walnut germplasm collection maintained at the USDA ARS National Clonal Germplasm Repository in Davis, CA which contains over 800 walnut genotypes representing 14 *Juglans* species. We collected open pollinated (OP) seeds from mother trees in this collection and screened the resulting seedlings for resistance to crown gall, and on a more limited scale, resistance to *Phytophthora* and root lesion nematodes. We narrowed our focus to *J. microcarpa* mother trees because this species provided a higher incidence of disease resistant OP progeny than other *Juglans* species. We have crossed the most promising *J. microcarpa* mother trees with pollen from *J. regia* to generate a breeding population on which we performed Genotype-by-Sequencing analysis in parallel with disease resistance phenotyping for the three pathosystems mentioned above. Using this information, along with genetic and physical maps of the parents, we are in the process of mapping QTL's associated with disease resistance. In the case of crown gall, a major QTL has been identified. Recent streamlining of our root lesion nematode screening efforts have established that two years of field observations are required to identify resistant and tolerant genotypes. In addition, further monitoring of these genotypes is necessary to determine the stability of nematode resistance and tolerance. Finally, putative disease resistant walnut genotypes which have emerged from our screening pipelines are being examined in large scale field trials to determine their commercial viability.

SUSCEPTIBILITY OF CALADIUM (*CALADIUM* × *HORTULANUM*) CULTIVARS TO *MELOIDOGYNE ARENARIA, M. ENTEROLOBII, M. FLORIDENSIS*, *M. INCOGNITA,* AND *M. JAVANICA*. **Kokalis-Burelle, Nancy[1], J.A. Brito[2], and R.D. Hartman[3].** [1]USDA, ARS, U.S. Horticultural Research Lab, 2001 South Rock Rd., Ft. Pierce, FL 34945, [2]Florida Department of Agriculture and Consumer Services Division of Plant Industry, Gainesville, FL 32608, [3]President & CEO of Classic Caladiums, LLC, 1315 State Road 64 W, Avon Park, FL 33825.

There is no known root-knot nematode (*Meloidogyne* spp.) resistance in caladium (*Caladium* × *hortulanum*), an ornamental foliage crop grown from tubers, but cultivars have been reported to differ in their level of susceptibility. Research was conducted to assess the relative susceptibility of seven widely grown caladium cultivars to species of *Meloidogyne* which commonly occur in the southeastern U.S., where caladium cultivars are commonly planted in commercial and residential landscapes. Root-knot nematode species tested were *M. arenaria, M. enterolobii* (=*M. mayaguensis*), *M. floridensis, M. incognita,* and *M. javanica*. All of the caladium cultivars tested were susceptible to galling by all species of *Meloidogyne* tested; however *M. javanica* had the least severe galling. *Meloidogyne enterolobii* produced high numbers of eggs/g fresh root on all cultivars tested, with cv. Freida Hemple having the highest number (14,799 eggs/g fresh root). *Meloidogyne javanica* also reproduced at a high level on most cultivars tested. Overall, the number of eggs of *M. arenaria, M. floridensis* and *M. incognita* were low on all caladium cultivars tested. *M. javanica* was isolated from caladium roots in high numbers regardless of cultivar. *M. incognita* had low numbers of J2 isolated from soil of all cultivars. The high level of reproduction of *M. enterolobii*, and the high rate of isolation of *M. javanica* from roots, as well as the low rate of isolation of *M. incognita* from soil are not reflected in gall ratings where *M. javanica* ratings were low but high numbers of eggs and J2 were present in roots. An increased understanding of cultivar susceptibility levels and the reproductive capacity of common root-knot nematode on caladium under various environmental conditions is needed to better manage nematode infested planting sites and improve caladium growth.

ADVANCEMENT IN BIOCONTROL RESEARCH: A CELLULAR LEVEL APPROACH TO STUDY TROPHIC INTERACTIONS. **Kooliyottil, Rinu, and L.M. Dandurand.** Department of Plant Soil and Entomological Sciences, University of Idaho, Moscow, ID 83844.

Several species of plant-parasitic nematodes (PPNs) are reported worldwide that can cause major damage to a broad range of agricultural crops. Due to the regulatory measures on chemical nematicides and lack of resistant varieties against most of the economically important PPNs, alternative strategies are urgently required. Nematophagous microorganisms are the natural enemies of nematodes, and they are potential candidates to control nematode pests. Biocontrol microbes have several mechanisms to control PPNs such as nematode trapping loop like appendages, production of virulence factors, and induction of resistance in plants. This presentation will focus on the technological advancement in microscopy and cell specific transcriptome to study plant-microbe and microbe-microbe interactions at cellular level. Non-destructive imaging techniques have been introduced recently to observe PPNs and biocontrol agents at very early to late in the parasitism stages. Endophytic nature of a biocontrol fungus *Chaetomium globosum* and its interaction with fluorescently labelled potato cyst nematode *Globodera pallida* in potato roots have been observed in our laboratory. Further advancement in these techniques have evolved to isolate cell specific RNA from the infected plant cells using microaspiration techniques. We propose the possibilities of these techniques to explore the multitrophic interactions at cellular level using advanced omics based methodology.

EARLY INFECTION TRANSCRIPTOME ANALYSIS OF *GLOBODERA PALLIDA* INFECTED IN THE SUSCEPTIBLE *SOLANUM TUBEROSUM* AND RESISTANT *SOLANUM SISYMBRIIFOLIUM*. **Kooliyottil, Rinu[1], L.M. Dandurand[1], J.C. Kuhl[1], A. Caplan[1], F. Xiao[1], B. Mimee[2], and J. Lafond-Lapalme[2].** [1]Department of Plant Soil and Entomological Sciences, University of Idaho, Moscow, ID 83844, [2]Agriculture and Agri-Food Canada, Horticulture Research and Development Centre, 430 boul. Gouin, St-Jean-sur-Richelieu, Quebec, Canada J3B 3E6.

The pale cyst nematode (PCN, *Globodera pallida*) is a devastating pest and one of the most economically important plant parasitic nematodes of potato. By contrast, when *G. pallida* infects *Solanum sisymbriifolium*, localized cell death occurs as early as 24-48 h post infection, and nematode development halts thereafter. In the present study, transcriptome analysis of *G. pallida* juveniles infected in *S. tuberosum* and *S. sisymbriifolium* at 24 h post infestation was performed. Infective second stage juveniles of *G. pallida* were inoculated on the roots of *S. tuberosum* and *S. sisymbriifolium*. After 24 h, the nematodes infected in the plant roots were recovered, frozen in liquid nitrogen and stored at -80 °C. RNA was extracted using a magnetic bead based method and the RNAseq libraries were constructed and then sequenced on an Illumina HiSeq 4000 platform. Sequencing reads were processed through quality control and read trimming, and aligned to the *G. pallida* genome. Differential expression analysis was performed using the Tuxedo pipeline. A total of 21,989 *G. pallia* genes were found from the mapped reads. Out of these, 41 showed significantly different expression values. Among this set, 12 were upregulated in *S. tuberosum* and 29 were upregulated in *S. sisymbriifolium*. Out of these twelve genes, three codes for secretory proteins; one is homologues to effector gene Rbp-4, the second one is an uncharacterized protein with a signal peptide, and the third one is an ortholog of a *Globodera rostochiensis* effector belonging to the1106 effector family. *In-planta* studies are in progress to study the virulence characteristics associated with these genes.

*GLOBODERA PALLIDA* EFFECTOR RHA1B MANIPULATES PLANT IMMUNITY THROUGH ITS E3 UBIQUITIN LIGASE ACTIVITY. **Kud, Joannna, W. Wang, Y. Fan, A. Duarte, L.M. Dandurand, and F. Xiao.** Department of Plant, Soil and Entomological Science, University of Idaho, Moscow, ID, 83843, USA.

*Globodera pallida* penetrates plant root system to establish a permanent feeding site, the syncytium, to gain access to nutrients inside plant cells. The development of this long-term biotrophic relationship with a host heavily relies on effector proteins injected into host cells though stylet to suppress plant innate immunity. Up to date, the large scale genomic and transcriptomic analysis have identified many potential effectors, yet, very few of those candidate virulence genes have been characterized in detail. Here, we report a comprehensive study of novel nematode effector, *Gp*RHA1B. The *Gp*RHA1B has N-terminal signal peptide, is highly upregulated in the early parasitic stage and its transcript localizes into secretory glands. Therefore, *Gp*RHA1B meets all the typical criteria for an effector protein. When transiently expressed in *Nicotiana benthamiana* leaves, *Gp*RHA1B effectively suppressed the defense-related programmed cell death (PCD) triggered by a potato Gpa2 protein conferring resistance to some isolates of *G. pallida* as well as by other CC-NB-LRR type proteins essential for viral (Rx), bacterial (Prf), and oomycete (Rpi-blb1) immunity. Moreover, *Gp*RHA1B interfered with pathogen associated molecular pattern (PAMP)-triggered immunity (PTI) in the plant, as manifested by inhibition of flg22-induced PTI signaling. Interestingly, the amino acid sequence suggests that *Gp*RHA1B is a RING-type E3 ubiquitin ligase. Both *in vivo* and *in vitro* ubiquitination assay confirmed predicted enzymatic activity. Significantly, *Gp*RHA1B promoted degradation of at least two tested resistance proteins, Prf and Gpa2. Consistently, the E3 ubiquitin ligase-deficient mutant *Gp*RHA1B $^{C135S}$ no longer suppressed PCD or promoted degradation of Gpa2, suggesting that enzymatic activity of *Gp*RHA1B is indispensable for its virulent activities. To our knowledge, *Gp*RHA1B is the first identified eukaryotic virulent effector carrying E3 ubiquitin ligase activity. Our biochemical analysis shed light on the *Gp*RHA1B mode-of-action during parasitism where *Gp*RHA1B manipulates host UPS to suppress plant immunity though targeting import defense-related proteins for degradation.

REPRODUCTION AND PATHOGENICITY OF NATIVE ISOLATES OF *ROTYLENCHULUS RENIFORMIS,* FROM LOUISIANA ON SOYBEAN. **Kularathna, Manjula[1], C. Overstreet[1], E.C. McGawley[1], S. Stetina[2], C. Khanal[1], F.M.C. Godoy[1] and D.M. Xavier-Mis[1].** [1]LSU Agricultural Center, Department of Plant Pathology and Crop Physiology, Baton Rouge, LA 70803, [2]USDA Agricultural Research Service, Stoneville, MS 38776-0345.

The reniform nematode (*Rotylenchulus reniformis)* is one of the major pests on both soybean and cotton in the south. Although there are resistant soybean cultivars available, this resistance may not be consistent with different geographical isolates of the pathogen. Experiments were conducted to evaluate soybean responses to indigenous isolates of the reniform nematode in Louisiana on commercial cultivars and resistant germplasm lines. Microplot and greenhouse experiments were conducted during 2016 and 2017 to evaluate the comparative reproduction and pathogenicity of isolates of *R. reniformis* isolated from West Carrol (WC), Rapides (RAP), Tensas (TEN) and Morehouse (MOR) parishes of Louisiana. Prior to the experiments single egg-mass populations of each geographic isolate were increased on tomato under greenhouse condition. Data from full-season microplot studies, averaged over 2 trials, showed significant differences of the nematode in both reproduction and pathogenicity with the commercial cultivars REV 56R63, Pioneer P54T94R, and Dyna-Gro 39RY57. There was a significantly lower population density with the isolate from MOR representing a 46% reduction in numbers compared to the isolate from the WC parish. The MOR isolate was also the most pathogenic and resulted in soybean plant and pod weights 30 and 44% lower, respectively compared to the noninoculated. Data from 60 day greenhouse experiments averaged over two trials reflected a similar trend to that of the microplot trials. In these trials the susceptible cultivar Progeny P4930LL and the resistant germplasm lines PI 90763 and PI 548316 were tested together with the same cultivars used in the microplot trials. Similar to the microplot trials, the MOR isolate had the lowest level of reproduction with a 33% reduction in life stages in soil compared to that of WC, the isolate with the greatest level of reproduction. This trend in reduction was more evident when determining the numbers of eggs per root system with a 50% reduction from MOR compared with WC. In microplot and greenhouse trials, the soybean cultivar REV 56R63 had a significant reduction in reniform numbers in soil compared to cultivars Pioneer P54T94R, and Dyna-Gro 39RY57 by contributing to a 45% and 70% reductions in soil density respectively. In the greenhouse trials REV 56R63 showed a pronounced resistant against all nematode isolates when compared to the moderately resistant germplasm line PI 548316. The resistant germplasm line PI 90763 was able to hold its resistance compared to tested cultivars and germplasm lines against all isolates of the reniform nematode. Data from these experiments add further evidence to the contention that there are virulence phenotypes of *R. reniformis.*

GENDER DIFFERENCE IN LESION FORMATIONS BY *PRATYLENCHUS PENETRANS.* **Kutsuwa, Kanan and A.E. MacGuidwin.** Plant Pathology Dept., University of Wisconsin-Madison, Madison, WI 53706.

Necrotic lesions, the hallmark symptom caused by *Pratyelnchus penetrans,* develop soon after infection. Studies on infection and egress behavior by *P. penetrans* showed males spend less time inside roots than females. The objective of our study was to verify and quantify the observation that lesions induced by females are more extensive than lesions caused by males. Experiments were conducted in vitro using *Pisum sativum* and *P. penetrans* cultured in vitro. Each experimental unit was a 3-cm long root radical excised from ''Early Alaska'' pea placed on water agar in a Petri dish. Two treatments, 40 adult

males or 40 fourth stage juvenile females, were placed 0.5 cm away from the root segment. Four replications of each treatment were processed according to a randomized block design and the experiment was repeated three times. Fourth stage juvenile females were molted to the adult stage during the experiment. The number of lesions, and individual and total lesion length were recorded daily. After 14 days, the roots were stained to count the number of nematodes inside. The data were analyzed for each experiment using a two-way ANOVA with time as a repeated measure factor. Data from the three experiments were combined, and experiment was treated as a random effect nested within replications. Lesions were first observed two and three days after inoculation for females and males, respectively. There were significant main effects of gender and time on total lesion lengths, average lesion lengths and numbers of lesions in the three experiments. The interactions of the two main effects were also significant for total lesion lengths and numbers of lesions, but not for average lesion lengths. The average lesion lengths for female and male generally peaked at 6 to 7 days after inoculation which then plateau. The total lesion lengths and lesion numbers on female-inoculated roots increased throughout each experiment, but the average length of individual lesions did not. The formation of new lesions ceased and the total lesion length plateaued on male-inoculated roots before each experiment was terminated. The final lesion lengths, average lesion lengths, and numbers of lesions on male-inoculated roots were reduced 69%, 33%, and 55% respectively, as compared to female-inoculated root at 14 days after inoculation. The final number of nematodes per root was not different for females versus males, indicating that the difference was not related to nematode population densities in roots. Studies are in progress to explain these results by comparing the feeding behavior of male versus female *P. penetrans*.

*HETERODERA GLYCINES* BIOTIN SYNTHASE (HgBioB) GENE MAY PLAY A ROLE IN SOYBEAN CYST NEMATODE HOST RANGE. **Kwon, Khee –Man and K.N. Lambert.** Department of Crop Sciences, University of Illinois, Urbana, IL 61801.

*Heterodera glycines*, the soybean cyst nematode (SCN), is a plant-parasitic nematode capable of manipulating and parasitizing host plants. Many studies have shown that the nematode has acquired genes through horizontal gene transfers (HGTs). For example, a bioinformatics screen of the *H. glycines* genome has shown that genes involved in vitamin B (vitamin $B_1$, $B_5$, $B_6$, and $B_7$) biosynthesis have been acquired through HGTs. Recently, an allelic imbalance analysis was used to associate single nucleotide polymorphisms with SCN genes that might be involved in virulence, the ability of a nematode to reproduce on resistant plants. This analysis revealed that *H.glycines* biotin synthase (*HgBioB*), the gene responsible for producing vitamin $B_7$, contained sequence polymorphisms between avirulent and virulent inbred SCN strains and was associated with the virulence phenotype. Here we report that avirulent and virulent nematodes produce biotin synthase with different enzyme activity. Complementation studies using BioB deficient *Escherichia coli* with *HgBioB* from both avirulent and virulent SCN alleles confirmed that avirulent nematodes produce an active enzyme while virulent ones synthesize an inactive form. Moreover, sequencing results from different SCN field populations showed that they all contained the inactive biotin synthase. Since not all field populations of SCN were virulent, this data indicates that *HgBioB* may not play a role directly in SCN virulence. Instead, *HgBioB* may play a role in SCN host range because the avirulent SCN population used in the study had a broader host range than most SCN, in that it was capable of growing on tomato. Future studies will include determination of biotin synthase enzyme activity from other SCN populations that grow on tomato as well as other closely related cyst nematodes such as *H. schachtii*, the sugarbeet cyst nematode. Understanding the exact role of *HgBioB* in host-nematode interactions may lead to the development of more durable resistant cultivars, which will aid in controlling these damaging pathogens.

SHARING INFORMATION ACROSS BOUNDARIES TO DELIVER A COMMON MESSAGE ON MULTIPLE PLATFORMS & PRESERVE KNOWLEDGE. **LaForest, Joseph.** University of Georgia, 4601 Research Way, Tifton, GA 31793.

Political boundaries have no bearing on the activity of pests or diseases and frequently require extension personnel to find solutions to pull together information from multiple partners. This task becomes challenging when the needed information is not readily available, requires significant processing for consumption or has been lost. This talk covers lessons learned in facilitating exchange of data. We will also discuss current tools for aggregating information, verifying data, delivering compiled information in real-time to diverse audiences, and providing a source of preserved knowledge for future extension personnel.

EFFECTS OF RESISTANT OR SUSCEPTIBLE TOBACCO (*NICOTIANA TABACUM*), EASTERN BLACK NIGHTSHADE (*SOLANUM PTYCHANTHUM*), AND LITCHI TOMATO (*SOLANUM SISYMBRIIFOLIUM*) ON REPRODUCTION OF THE TOBACCO CYST NEMATODE, *GLOBODERA TABACUM*. **LaMondia, James A.[1] and L.-M. Dandurand[2].** [1]The Connecticut Agricultural Experiment Station Valley Laboratory, PO Box 248, Windsor, CT 06095, [2]University of Idaho, Moscow, ID 83844.

The influence of nematode-resistant or susceptible cigar wrapper tobacco (*Nicotiana tabacum*), eastern black nightshade (*Solanum ptychanthum*), and Litchi tomato (*Solanum sisymbriifolium*) on reproduction of the tobacco cyst nematode, *Globodera tabacum* (TCN), was investigated in field microplots over two years. Sixty-five microplots 1-m-diam, naturally

infested with various densities of TCN, were transplanted with nematode-susceptible shade tobacco (cv. '8212' in 2015, and 'O-40' in 2016), nematode-resistant broadleaf tobacco cv. 'B2', or Litchi tomato. In 2016, treatments were expanded to include eastern black nightshade and a cultivated fallow. TCN densities were determined before planting and again after harvest by sampling each microplot with 10 cores 1.5-cm-d to 15-cm depth. Soil was dried and extracted using a modified Fenwick can. Cysts were crushed and the number of viable encysted J2 per $cm^3$ soil determined. Nematode reproduction as determined by the ratio of the final (Pf) to initial (Pi) populations varied between treatments (P = 0.003). In 2015, Pf/Pi ratios were 2.89, 0.38 and 0.14 for susceptible tobacco, resistant tobacco and Litchi tomato, respectively. All three plants were significantly different from each other (P = 0.05). In 2016, Pf/Pi ratios were highest for eastern black nightshade (6.64) and susceptible tobacco (2.84), which were different from fallow (0.56), resistant B2 tobacco (0.32) and Litchi tomato (0.20). These results are consistent with previous research that Litchi tomato, *S. sisymbriifolium*, stimulates tobacco cyst nematode hatch better than resistant or susceptible tobacco but unlike eastern black nightshade, does not allow significant nematode reproduction in roots, indicating that it may be an effective trap crop for management of *G. tabacum*. In addition, *G. tabacum* may be useful as a substitute model for the quarantined pathogen *Globodera pallida* for trap cropping with *S. sisymbriifolium* under field conditions.

AN INTERDISCIPLINARY ASSESSMENT OF INTEGRATED NEMATODE-SOIL HEALTH MANAGEMENT FOR SMALLHOLDER POTATO FARMING SYSTEMS IN THE WESTERN HIGHLANDS OF GUATEMALA. **LaPorte, Patricia[1], B. Sipes[1], H. Melakeberhan[2], C. Chan[1], A. Sanchez-Perez[3], and A. Sacbaja[3].** [1]University of Hawaii at Mānoa, Honolulu, HI, [2]Michigan State University, East Lancing, MI, and [3]Universidad de San Carlos de Guatemala, Guatemala City, Guatemala.

Improving soil health and developing sustainable nematode management strategies are major challenges in achieving global food security, particularly in emerging economies. For example, smallholder potato farmers of the Highlands of Guatemala face substantial yield losses from the potato cyst nematode (PCN) and poor soil health. However, many challenges exist in delivery and adoption of technologies for soil health and nematode management within and across diverse socio-cultures. This project, funded by the Horticulture Innovation Lab, seeks to assist farmers in adopting integrated nematode-soil health management technologies to achieve sustainable yields that provide enhanced income and food security. Mental modeling, a social science approach to understanding socio-cultural-economic-environmental behavior, where farmers/stakeholders are part of designing the experiment is a critical component of this project. We have initiated a baseline study on Andisol and Mollisol soils occupied by Mam and Quiche peoples where three focus groups of farmers cited potato yield decreases of 50% over the past 20 years, from 5.4 $kg/m^2$ to 2.7 $kg/m^2$. Potato production is mainly for local markets or household consumption, with around 25% of the potato harvest exported to El Salvador and Honduras.P otatoes are often the main cash crop, after black beans, for the smallholder households in Guatemala. Potato is generally rotated with maize and farmers tend to use their own seed rather than purchasing certified seeds. Most farmers incorporate organic matter – either, forest leaf litter, chicken litter, or composted animal manure. However, some farmers associate the use of chicken litter with the spread of PCN. While rotation and incorporation of organic matter has benefits, other agricultural practices of the smallholder farmers exacerbate the PCN and poor soil health problems. Currently, returns to yield decrease as the smallholder farmers increase inputs such as fertilizer ($P>0.09$). The costs of fertilizer is not sufficiently enhancing potato yield. The data on smallholder potato production suggests decreasing returns to scale so farmers are not cost efficient. Experimental treatments to improve soil health and manage PCN were co-designed with stakeholders and have resulted in the evaluation of treatments consisting of composted chicken manure and biological controls for PCN. Through the mental modeling approach, the team will be map how potential stakeholders view the relationships of the soil health, economics and nematode management outcomes of the experiments that they co-designed as well as scalability across soil groups and socio-cultures in Guatemala.

THE IDENTIFICATION OF GENES HAVING DEFENSE ROLES TO NEMATODES THROUGH A FUNCTIONAL DEVELOPMENTAL GENOMICS SCREEN. **Lawaju, Bisho Ram[1], B.T. McNeece[2], S.R. Pant[3], K. Sharma[2], P.M. Niraula[2], W.A. Aljaafri[1], G.W. Lawrence[1], K.S. Lawrence[4], V.P. Klink[2].** [1]Department of Biochemistry, Molecular Biology, Entomology & Plant Pathology, Mississippi State University, Mississippi State, MS, 39762, [2]Department of Biological Sciences, Mississippi State University, Mississippi State, MS, 39762, [3]Department of Plant Pathology & Microbiology, Texas A&M AgriLife Research & Extension, Weslaco, TX, 78596, [4]Department of Entomology & Plant Pathology, Auburn University, Auburn, Al, 36849.

RNA has been isolated from *Glycine max* (soybean) root cells undergoing the process of defense to *Heterodera glycines* (soybean cyst nematode). The RNA has been used in gene expression analyses. The procedure has led to the identification of candidate resistance genes. A gene testing platform has been developed to functionally test these genes. The procedure has examined hundreds of genes with some functioning effectively in defense. The analysis has demonstrated the importance of various cellular processes to defense and has identified genes that previously had no known role in defense.

PLANT-PARSITIC NEMATODE DISTRIBUTION AFTER NEEM CAKE AMENDMENT ACROSS A GUAVA ORCHARD IN BRAZILIAN SEMIARID REGION. **Leitão, Diego Arruda Huggins de Sá, A.K.S. Oliveira, D.B. Castro, A.A.A. Montenegro, and E.M.R. Pedrosa.** Agricultural Engineering Dept., Federal Rural University of Pernambuco, Recife, PE, Brazil 52171-900.

Plant-parasitic nematodes pose a great threat to worldwide food security, hence new alternatives to nematode management need to be evaluated. Although the suppressive effect of organic matter amendment has been reported under greenhouse conditions, little is known about the effect at field scale. Additionally, the use of geostatistics to map nematode spatial distribution has been advantageous for site-specific nematode management. Therefore, we aimed to map the spatiotemporal distribution patterns of plant-parasitic nematodes and soil physical attributes after neem cake amendment. A study was carried out from May to November 2013 in a family-farming guava field in the semiarid region of Pernambuco, Brazil. The study site consisted in 0.42 ha (61 x 69.8 m) with approximately 400 guava seedlings (cv. Paluma) grafted six months prior to the experiment. A regular 48-point sampling grid was delimited with 10 m spacing between points; each sampling point represented one guava tree. Neem cake was amended in May and August 2013 at the rate of 1 kg per tree, following the canopy's projection at 0.20-0.40 m. Soil samples were collected three times: T1) before neem cake amendment; T2) three months after the first amendment; and T3) three months after the second amendment, in order to perform nematode and physical analysis. Plant-parasitic nematodes were identified at genus or family level. Soil physical attributes comprised bulk density (BD), total porosity (TP), water content (WC), electrical conductivity (EC) and particle size distribution. Descriptive and geostatistical analyses were performed after log(x+1) and 1/x transformations for nematode and EC data respectively, with further generation of contour maps through the fitting of data semivariances to theoretical models. Data normality was observed for soil physical attributes throughout the experiment, except for clay content, and for endoparasitic and total plant-parasitic nematodes in T1. Nematode and physical attributes were best fitted to spherical or Gaussian models. Range values for *Meloidogyne* and *Rotylenchulus* increased from 23,36 and 14,64 m to 37,45 and 38,99 m, respectively, after neem cake amendment. On the other hand, soil physical attributes range values decrease after amendment, indicating that they were less homogenously distributed throughout the area. In contrast to Criconematidae, higher population densities of *Meloidogyne* were found in lower BD and higher PT regions in T3. *Rotylenchulus* spatiotemporal behaviour did not depend on soil physical attributes.

EFFECT OF CHEMICAL INSECTICIDES ON SURVIVAL AND INFECTIVITY OF ENTOMOPATHOGENIC NEMATODES. **Li, Chunjie[1], X. Zhou[2], J. You[1], Y. Yu[2], and C. Wang[1].** [1]Key Laboratory of Mollisols Agroecology, Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences, Harbin 150081, China; [2]Shandong Key Laboratory of Plant Virology, Institute of Plant Protection, Shanong Academy of Agricultural Sciences, Jinan 250100, China.

Entomopathogenic nemtodes (EPNs) as an environmentally-friendly biocontrol agent can suppress various economically important insect pests. To increase better control and reduce cost, EPNs may be combined with low toxic insecticides. However, insecticides often have negative impact on EPN survival and EPN infectivity to insects. Therefore, the compatibility of EPN species/strains with insecticides including dosage effect are required to be established and the mechanism resulted in synergistic control effect need to be understood. In this study, one important insect on Chinese chive, root gnat (*Bradysia odoriphaga* Yang et Yang) was used to study the compatibility with *Steinernema carpocapsae*-All (Sc-All) including host-seeking behavior, EPN survival and infectivity, and application dosage of both EPN and insecticide under laboratory conditions. The results showed that exposures of Sc-All to insecticide phoxim with the recommended concentration of field application resulted in 16.2% and 43% corrected mortality at 24 and 48 hr, respectively, but no effect on Sc-All when using other three insecticides, matrime, imidacloprid and chlorpyrifos. Half dosage of Sc-All (200IJs/larva) in the presence of the four insecticides with 1/10 field recommended application concentration showed synergistic effect on the corrected mortality (range from 18.5% to 100%) of chive gnat at 48 hr compared with the corresponding insecticide and EPN alone. Of them, the corrected mortality of *B. odoriphaga* reached up to 100% at early 24 hr after the combination of Sc-All and imidacloprid. While only 9.4% and 0 corrected mortality were detected when exposed to same amount of imidacloprid and Sc-All alone, respectively, at 24 hr. Meanwhile, we found Sc-All host-seeking at 30 min and penetrating rate to the chive gnat at 24 hr after the assay was significantly higher in the presence of the insecticides than in the water only, suggesting high attraction and penetrating rate increase the insect mortality. Thus, combinations of low levels of both insecticides and EPNs resulting in high mortalities of the chive gnat will reduce cost and enhance the control efficacy of EPN as biocontrol agents.

PLANT HORMONE MANIPULATION DURING RENIFORM NEMATODE (*ROTYLENCHULUS RENIFORMIS*) PARASITISM AND EFFECTS ON UPLAND COTTON (*GOSSYPIUM HIRSUTUM*) ROOT ARCHITECTURE. **Li, Wei[1], P. Agudelo[1], R. Nichols[2], and C.E. Wells[3].** [1]Department of Plant and Environmental Sciences and [3]Department of Biological Sciences, Clemson University, Clemson, SC, 29634. [2]Cotton Incorporated, Cary, NC, 27513.

Reniform nematode (*Rotylenchulus reniformis,* RN) parasitism on upland cotton (*Gossypium hirsutum*) roots involves modification of pericycle cells where lateral roots initiate. Our objectives were (1) to assess how reniform nematode infection affects lateral root formation in cotton and (2) to compare root architectural changes with differential gene expression during

RN parasitism. Total root length, fork density, and fractal dimension were measured with a WinRHIZO root scanner across a 12-day infection time course (3, 9 and 12 days after inoculation, DAI). Transcriptomes of RN-infected and uninfected cotton roots from a split root system were sequenced and analyzed over the same time course. RN-infected cotton root systems had significantly higher fractal dimensions and fork densities, as well as longer total root length ($p<0.05$). A similar effect of RN infection on cotton roots was observed in germination pouch cultures. One hundred forty-eight differentially expressed (DE) genes and 17 enriched gene sets had potential functions in the modification of root system architecture (FDR<0.05). Expression profiles of selected transcripts indicated three distinct developmental stages of reniform nematode syncytia: an initial peak of up-regulation was observed at 3 DAI (initiation), followed by the highest number of DE transcripts at 9 DAI (expansion) and the lowest number of DE transcripts at 12 DAI (maintenance). Differentially expressed genes and gene sets included transcripts putatively involved in auxin signaling and transport, root morphology, and manipulation of additional hormones. Transcriptomic data suggest that, at the syncytial initiation stage, auxin signaling pathways may be activated by enriched ubiquitin ligase complex and proteasome core complex assembly gene sets. Basipetal auxin transport may be interrupted by up-regulated ethylene and abscisic acid signaling pathways, resulting in auxin accumulation at the infection site. To expand syncytia, auxin polar transport (influx and efflux) and auxin diffusion may be modified to facilitate feeding site expansion from cell to cell. Auxin-inducible transcripts, including transcription factors (e.g. WRKY, and MYB) and root morphology regulators (e.g. LOB domain containing protein), were differentially expressed. At 12 DAI, depressed root cell cycler regulators (e.g. flowering-promoting factors and *EXORDIUM*) and DE auxin transporters were associated with syncytial maintenance. Our results suggest that, during nematode parasitism on cotton roots, reniform nematodes co-opt plant hormone pathways. The changes in auxin signaling and transport to establish feeding sites in pericycle cells may result in modified root system architecture.

MATERNAL STRESS REDUCES THE SUSCEPTIBILITY OF *MELOIDOGYNE ARENARIA* PROGENY TO *PASTEURIA PENETRANS*. **Liu, Chang[1] and P. Timper[2]** . [1] Department of Plant Pathology, University of Georgia, Tifton, GA 31794, [2]USDA ARS, P.O. Box 748, Tifton, GA 31793.

*Pasteuria penetrans* is an obligate parasite of *Meloidogyne* spp. Endospores of *P. penetrans* attach to the cuticle of the second-stage juvenile (J2) and the bacterium completes its life cycle in the mature female nematode; infected females are filled with millions of endospores and produce few to no eggs. Research with *Pasteuria ramosa* and water fleas has shown that a poor maternal environment increases resistance to *P. ramosa*. Therefore, we hypothesized that female nematodes that were under stress would produce progeny that were more resistant to *P. penetrans*. In the greenhouse, we created two environments for *Meloidogyne arenaria*: stressed as a result of crowding and non-stressed. The stressed treatment was inoculated with 5000 J2 per pot and the foliage of the host plant (eggplant) was pruned to reduce root growth. The non-stressed treatment was inoculated with 1000 J2 and no pruning was done. Two months after inoculation, the soil was sampled for the presence of males (an indication of population stress) and eggs were extracted from females in the stressed and non-stressed treatments. The eggs were hatched to obtain J2, and the J2 were exposed to *P. penetrans* endospores to determine susceptibility to spore attachment. Juveniles with spores were also inoculated onto eggplant to determine the percentage of progeny which were infected by the bacterium. There were six replicates of each treatment and the experiment was conducted twice. Stressed treatments contained 3.4 males/100 $cm^3$ of soil and non-stressed treatments contained no males. There was no difference in attachment of endospores to progeny from stressed and non-stressed environments, both treatments averaged 5.8 spores/J2. However, the percentage of females infected by *P. penetrans* was lower in progeny from stressed (8%) than from non-stressed (18%) mothers. These findings suggest that populations of *Meloidogyne* spp. that reach the carrying capacity of their host plant, as often happens at the end of the season, may generate progeny which are less susceptible to infection by *P. penetrans* than are populations not competing for nutrition.

NEMATODE-SUPPRESSIVE POTENTIAL OF DIFFERENT DIGESTATES TO *MELOIDOGYNE INCOGNITA* AND *HETERODERA SCHACHTII*. **Liu, Ke[1], A. Edalati[2], R. Zhang[2], and A. Westphal[1].** [1]Department of Nematology, University of California, Riverside, Riverside, CA 92521, USA [2]Department of Biological and Agricultural Engineering, University of California, Davis, Davis, CA 95616.

Plant-parasitic nematodes can damage almost any crop, and their management primarily relies on plant resistance, crop rotation, and the use of nematicides. Concerns for air and water quality, and for human health lead to restrictions of chemical management options and the need for alternative approaches. For example, amendments with anaerobically digested maize silage had previously been shown to have nematode suppressive potential. The objective of this study was to determine whether differences in nematode-suppressing potential exist among digestates and their processing stages either from a single-stage mesophilic dairy manure digester or from two thermophilic digesters: (1) filtered dairy manure digestate, (2) liquid dairy manure digestate with ammonia mostly removed, (3) food waste digestate, (4) liquid food waste digestate with ammonia mostly removed, (5) liquid food waste digestate, and (6), (7) food waste hydrolysates from the two different digesters. In a radish bioassay, digestate rates of 0.008-, 0.04-, 0.2-, and 1- fold of 1 ml / 35 ml soil were added. Amendment of digestates 3, 5, and 6 at 1 ml / 35 ml of soil reduced *Heterodera schachtii* nematode root penetration compared with the

non-amended control. In a watermelon bioassay, the same amendment concentration series was used. Amendments with digestates 3, 5, and 6 at 1 ml / 35 ml of soil reduced the number of *Meloidogyne incognita* egg masses on the roots compared with the non-amended control. In a microplot experiment with *M. incognita*, amendment with digestate 5 (1 ml / 112 ml soil) increased the radish marketable yield compared with the non-amended control. These experiments illustrate that digestates have nematode-suppressive potential that may differ between digestate sources.

COMBINATION OF MICROBIAL ANTAGONISTS AND A SEED-DELIVERED NEMATICIDE MITIGATED ROOT-KNOT NEMATODE-CAUSED DISEASE IN TOMATO GREENHOUSE AND MICROPLOT TRIALS. **Loffredo, Angelo[1], J. Smith Becker[1], R. Fukui[2], and J.O. Becker[1].** [1]Department of Nematology, University of California, Riverside, CA 92521, [2]Faculty of Agriculture, Utsunomiya University, Utsunomiya, Tochigi, Japan.

Root-knot nematodes (rkn; *Meloidogyne* spp.) are a major disease-causing problem in California's tomato production. The regulatory restrictions on soil fumigants, lack of potent contact nematicides, and increasing spread of rkn populations that overcome the Mi-resistance gene led us to evaluate various microbial antagonists. Although female and egg parasites might be effective in reducing rkn nematode populations when females mature, typically they fail to reduce the initial root attack during the crop's most vulnerable early growth phase. In contrast, nematicidal seed coatings are ideally situated to protect young seedlings but typically diminish in efficacy after a few weeks. Repeated tomato (cv. Camone) greenhouse and microplot trials were conducted with *M. incognita*-infested sandy loam (1,000 rkn eggs/100 $cm^3$ soil) in a randomized complete block design with 5 replications. We evaluated the biological control agents *Pochonia chlamydosporia* (5,000 chlamydospores/g soil) and *Pasteuria penetrans* ($1x10^5$ endospores/g soil) as well as the nematicidal seed coating Avicta (0.3 mg a.i. abamectin/seed) alone and in combination. In the greenhouse trials, the combination of the two biologicals with the seed treatment reduced galling by two rating classes (Zeck scale 0-10) compared to the untreated control. We observed similar disease symptom reductions at the end of the 3-month microplot trials. The three-component combination was superior to individual treatments; total fruit weight increased by 55% and the number of marketable fruits increased by 64% compared to the untreated control. The results support our hypothesis that a combination of nematicidal seed coating and biological control agents might improve the weak aspects of each single method.

DISTRIBUTION AND ABUNDANCE OF *HETERODERA GLYCINES* AND OTHER PLANT PARASITIC NEMATODES FROM SOYBEAN FIELDS IN PARAGUAY. **Lopez-Nicora, Horacio[1], L.M. Pedrozo[2], C. Grabowski Ocampos[3], A.L. Orrego Fuente[3], E. Hahn Villalba[4], O.A. Guzman Piedrahita[1], and T.L. Niblack[1].** [1]Department of Plant Pathology, The Ohio State University, Columbus, OH, U.S.A., [2]Instituto Paraguayo de Tecnología Agraria, Caacupé, Paraguay, [3]Facultad de Ciencias Agrarias, Universidad Nacional de Asunción, San Lorenzo, Paraguay, [4]Facultad de Ciencias Agropecuarias, Universidad Católica, Itapúa, Paraguay.

Paraguay ranks sixth among soybean-producing countries in the world and fourth in soybean exports. Soybean is an important commodity for Paraguay's economy and a major field crop based on planted area. Currently, out of the seventeen Paraguayan departments, ten are considered important soybean production regions. In 2002, *Heterodera glycines*, the soybean cyst nematode (SCN), was reported for the first time in Paraguay from one soybean field. By 2008, the presence of *H. glycines* was confirmed in two additional departments. The objective of this study was to determine the presence, distribution, and abundance of SCN and other plant parasitic nematodes from soybean fields in major agricultural regions of Paraguay. During the 2014/2015 growing season, composite samples were collected from 300 soybean fields arbitrarily chosen across eight soybean-producing departments in Paraguay. Five departments included in previous surveys were revisited in this study; however, new soybean fields within each department were sampled. Samples were processed for SCN eggs and vermiform nematodes per 100 $cm^3$ soil with standard techniques. Plant parasitic nematodes were identified to genus level. SCN eggs per 100 $cm^3$ soil ranged from 40 to 1,320 and were detected in 21 samples from four departments. Of these samples, 14 were from the three departments from which SCN was previously reported. The nematode was detected in seven fields from a fourth department that had been included in past surveys but from which SCN was not confirmed. The four departments infested with SCN include more than 77 % of the land planted to soybean in Paraguay. Moreover, several plant parasitic nematode genera were identified in this study and are presented below in increasing order of prevalence: *Hoplolaimus* (range 0 to 120/100 $cm^3$ soil), *Criconemoides* (0 to 158), *Rotylenchulus* (0 to 1,800), *Meloidogyne* (0 to 360), *Trichodorus* (0 to 26), *Tylenchorhynchus* (0 to 680), *Scutellonema* (0 to 2,356), *Pratylenchus* (0 to 176), and *Helicotylenchus* (0 to 2,562). Among these genera, *Pratylenchus* and *Rotylenchulus* could be of major concern for soybean production in Paraguay because neighboring countries have reported significant soybean damage due to certain species in these genera. Identification to species of these two genera is underway. Information from this study highlights the spread of SCN to major soybean producing regions in Paraguay and the potential threat to soybean production due to plant parasitic nematodes. This study may aid local growers in developing sustainable economic and agricultural systems.

COMPARATIVE GENOMICS OF TWO LANCE NEMATODES: *HOPLOLAIMUS COLUMBUS* AND *H. GALEATUS*. **Ma, Xinyuan[1], V. Richards[2], J. Mueller[3], and P. Agudelo[1].**[1]Plant and Environmental Sciences Dept., Clemson University, Clemson, SC, 29634, [2]Biological Sciences Dept., Clemson University, Clemson, SC, 29634,[3]Edisto Research and Education Center, 64 Research Rd., Blackville, SC, 29817.

Here we report the draft genome sequences of two lance nematodes, *Hoplolaimus columbus* and *H. galeatus*. These migratory ecto-endo parasitic nematodes are of agricultural importance on field crops and turfgrasses, and have been reported as causing significant financial loss in the southeastern US. The project utilized a combined protocol including flow cytometry estimation of genome sizes, DNA extraction from single nematodes, whole genome amplification, and Next Generation Sequencing with Nextera XT pooled-library. Using Illumina MiSeq v3 kit, 56 million 300bp-reads were generated, yielding approximately 13 Gbp of high quality data (Q-score >30). The initial assembly using SPAdes 3.10.1 estimated a genome size for *H. columbus* of 250Mbp with 39.4% GC content, and 144Mbp for *H. galeatus* with 40% GC content. These genome sequences will be useful in identifying informative regions for building references and for developing genetic tools for species identification and phylogenetic investigations. Of special significance is the potential contributions to the elucidation of the origin of *H. columbus*, first reported from South Carolina, USA in 1963, but believed to be an introduced species. These genomes are also a tool that enables comparative genomic approaches to studying parthenogenetic reproduction and its role in speciation of lance nematodes.

IMPROVING SOIL CONDITIONS FOR ENTOMOPATHOGENIC NEMATODES WITH NO-TILL COVER CROPPING. **Marquez, Josiah, K.-H.Wang, B.S. Sipes, and Z. Cheng,** Department of Plant and Environmental Protection Sciences, University of Hawaii, Honolulu, HI 96822.

Entomopathogenic nematodes (EPNs) are promising biocontrol agents for insect pest management. Use of EPNs in Hawaii is challenged by quarantine restrictions and the failure of introduced EPN to persist in the field. This research focuses on enhancing indigenous EPN (*Heterorhabditis* sp.) populations in the field through conservation agriculture. We hypothesized that cover cropping followed by a no-till practice in Oxisol soil would provide a favorable environment for EPN via provision of organic mulch, reduced soil disturbance, improved water conservation, and possibly production of herbivore-induced volatiles (HIPVs) from cover crops. Two field experiments hereby referred to as Oil Radish (OR) Experiment and Black Oat (BO) Experiment, both repeated once, compared pre-plant treatments of 1) black oat (BO), *Avena strigose,* or oil radish (OR), *Raphanus sativus*, as cover crops in no-till plots, 2) bare ground (BG) followed by conventional tillage, and 3) conventional tillage followed by soil solarization (SOL) on abundance and infectivity of EPNs using mealworm larva (*Tenebrio molitor*) as a bait. Data were taken every other week throughout the 3 months of corn (*Zea mays*) growth. Indigenous EPNs recovered were identified by sequencing the ITS region and matched an undescribed species of *Heterorhabitis* in NCBI, labeled as H1, SGgj, SGmg3, and SSRKK15. Soil in BO and OR had higher volumetric soil moisture, field capacity, and soil organic matter than BG and SOL ($P < 0.01$) in all four trials. In addition, the nematode soil food web at termination of the cover crop, and monthly during corn growth were more mature and structured in BO and with a greater abundance of predators in OR than in BG and Sol ($P \leq 0.05$). No-till cover cropping appeared to provide an environment favorable for nematodes sensitive to soil disturbance which may include indigenous EPNs. In BO, EPN infectivity was greater ($P < 0.01$) than in BG and SOL based on field cage assays. BO may provide a habitat that enhances EPN infection. A multivariate canonical analysis conducted for data collected throughout the BO Experiment suggested that higher EPN infectivity had a negative relationship with thrips populations, suggesting that no-till cover cropping with black oats may have reduced thrips populations. EPN abundance in OR was greater than in BG ($P<0.01$) at cover crop termination, supporting the hypothesis that OR might have produced HIPVs that lured EPNs. However, no-till cover cropping with OR failed to increase EPN infectivity in the field during the corn growing season. This may be due to the lack of soil coverage over time, causing an unfavorable condition for infections in the field. Future research is needed to understand if HIPVs are involved in increasing ability of OR to attract EPNs, and if a mixture of OR and BO cover cropping can improve EPN infectivity.

HOST STATUS OF YELLOW AND PURPLE NUTSEDGE TO *MELOIDOGYNE GRAMINIS*. **Mendes, Maria de Lourdes, D.W. Dickson, and W.T. Crow.** Entomology and Nematology Department, University of Florida, Gainesville, FL 32611.

*Meloidogyne graminis*, the grass root-knot nematode, is among the most important nematode pathogens of bermudagrass (*Cynodon* spp.) and other turfgrasses in Florida, but its ability to infect common weeds is unknown. The objective this study was to evaluate the host status of yellow (*Cyperus esculentus*) and purple nutsedges (*C. rotundus*) to *M. graminis* under greenhouse conditions. The experimental design was completely randomized with five replications and three treatments, yellow and purple nutsedges, and a susceptible control 'Tifway' bermudagrass. Clay pots containing 1.2 liters of sand were seeded with six tubers each of either yellow or purple nutsedge, or sprigged with bermudagrass. Sixty days later each pot was inoculated with 4,000 second-stage juveniles (J2) of *M. graminis*. After 150 days the nematode population density was assessed along with fresh root and tuber weights. J2 were extracted from 100 $cm^3$ of soil using the sugar flotation method. Nematodes were extracted from the roots and tubers separately from the entire content of each pot using the Seinhorst mist

extraction method for a period of 72 hours. Ca. 15,000 and 16,000 J2 were extracted from purple nutsedge and bermudagrass, respectively, whereas only 1,700 J2 were extracted from yellow nutsedge. There was a 5-fold increase in number of J2 extracted from tubers of purple nutsedge vs. yellow nutsedge. In summary, the host status of purple nutsedge was equal to that of 'Tifway' bermudagrass.

EXAMINATION OF ROOT-KNOT NEMATODE RESPONSES TO HOST CIRCADIAN AND DIURNAL RHYTHMS. **Mishra, Shova, N. Abdelsamad, and P.M. DiGennaro.** Department of Entomology and Nematology, University of Florida, Gainesville, FL 32611.

Root-Knot nematodes (RKN; *Meloidogyne* spp.) are obligate plant-parasites with extensive host ranges, infecting over 2000 plant species. Successful parasitism of such a diverse genera of hosts implicates basal plant processes as mediators of the plant-nematode interaction. One of the most fundamental regulators of plant biology is light, manifesting as circadian and diurnal patterning of gene expression and phenotypes. Previous RNA-Seq analyses showed diurnal expression patterning of the meristematic maintenance gene, *KNOX1,* specifically displaying higher expression during the night in RKN feeding sites. *KNOX1* is a transcriptional regulator known to be involved in nodule and giant cell maintenance of RKN feeding sites. Other pathways highlighted by this study include single carbon metabolism, suggesting RKN feeding is not a continuous process. Light has been largely overlooked as a contributor to the plant-nematode interaction, and we hypothesize circadian and diurnal rhythms directly affect RKN parasitism, including host perception, penetration and feeding site initiation and maintenance. To investigate the possible roles of host circadian and diurnal patterning on RKN penetration, we conducted a time course experiment. Three-week-old *Medicago trancatula* Jemalong A17 seedlings were inoculated with 600 J2s, after 24-hours post inoculation roots were collected every 2-hour for a total number of 12 time points to quantify nematode penetration using qPCR and root staining assays. In addition, we studied the expression patterns of *M. trancatula* genes such as *KNOX1*, *PHAN*, *AGAMOUS*, and *XAP5* circadian timekeeper. We are also interested in the circadian patterning of gene expression changes in RKN and host in developed feeding sites. Using the previous RNA-Seq data set as a guide, we will map plant and nematode temporal transcriptional changes to better characterize the intimate host-pathogen interaction. This study will help us to illustrate on how changes in host biology, particularly changes due to light and dark cycle, affect the nematode parasitism and reveal a functional link between light regulated plant gene expression and nematode biology.

USING NEXT GENERATION SEQUENCING FOR HIGH THROUGHPUT IDENTIFICATION OF NEMATODES. **Mohammed, Ahmed[1], Melanie Sapp[2], Tom Prior[2], Gerrit Karssen[3] and Matthew Back[1].** [1]Harper Adams University, Edgmond, Newport TF10 8NB, UK, [2]Fera Science Ltd. Sand Hutton, York YO41 1LZ, UK, [3]National Plant Protection Organization, P.O Box 9102, 6700HC Wageningen, the Netherlands.

Species identification constitutes a key aspect of our understanding of life's diversity and the impact this has on ecosystem functioning. Nematodes represent one of the most species-rich and morphologically diverse groups of metazoans inhabiting both aquatic and terrestrial environments. In spite of this, knowledge of their diversity has been limited, often due to the difficulty in achieving species identification using morphological characters. In this study, four DNA markers: two regions of the 18S rRNA gene, a region of the 28S rRNA gene and the COI gene were evaluated for use in metabarcoding on an artificially assembled nematode community of known diversity and abundances. Using the sequence reads generated for one of the 18S rRNA markers, three commonly used open source high throughput sequence data analysis pipelines, mothur, qiime and usearch, were also compared for accuracy of the clustering and taxonomy assignment methods they use and the overall time taken to run the complete the analyses. Next generation sequencing, conducted on a mock nematode community, highly supported the utility of the two 18S rRNA gene markers for such high throughput identification. The COI mtDNA gene produced less coverage but was still able to correctly assign taxonomy to some of the molecular operational taxonomic units (OTUs). None of the targeted markers could estimate abundance of the different taxa in the mock community from the read numbers of their respective OTUs. Nonetheless, the 18S rDNA-based markers could still recover the majority of the sampled taxa and assign them to the correct identities through a BLAST search at a similarity cut-off of 97%. In terms of closeness of total number of OTU to predicted diversity, usearch and mothur performed significantly better than qiime. Recovery of sampled taxa was generally comparable for all three pipelines. Mothur took approximately 34 hrs to run the complete analysis, qiime took 3 hrs and 15 min with the same computing power. It took about 12 min for the complete analysis to run within usearch. In conclusion, while the 18S-based markers provided excellent coverage, their read frequencies as with those of the other markers deviated significantly from the actual abundance of the taxa. The analysis pipeline, usearch was overall the fastest and just as accurate as the other two.

BIOGEOGRAPHICAL PATTERN OF SOIL NEMATODE INDICATES A MID-ELEVATION DIVERSITY MAXIMUM ON MT.NORIKURA, JAPAN. **Moroenyane, Itumeleng[1], K. Dong[2], D. Kerfahi[2], K. Takahashi[3], N. Yamamoto[4], C. An[4], B. Tripathi[5], H. Cho[2] and J. Adams[2].** [1]Institut National de la Recherche Scientifique,Centre Institut Armand-Frappier, 531 boulevard de Prairies, Laval, Québec, H7V 1B7,[2]Department of Biological Sciences, College of Natural Sciences, Seoul National University,Seoul 151-742, South Korea, [3]Department of Biology, Faculty of Science, Shinshu

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Understaning biographical patterns of nematodes across along environmetal gradients has become increasingly important in a changing environment. Previously, studies have elucidated the taxonomic and diversity patterns of nematodes across different ecotypes in Japan. However, most of the studies have focused on commercially important nematodes, none have compared the biogeogrpahical patterns of free-living nematodes along elevation gradient. Here, we used a novel metagenetic approach to investigate the free-living nematode community structure on Mt. Norikura. We surveyed nematodes along ~2200m elevation range by sequencing the 18S rRNA gene on the Illumina MiSeq platform. Similar to previos studies from this system, we found that nematod diversity and abandace showed correlation with elevation, and maximum diverity in mid-elevations. Although, elevation alone in the context of mid-domain effect can predict the observed biogeographical aptterns of soil nematode communities, however total soil nitrogen concentration and mean annula temperature were the best predictors of nematode diversity. Moreover, soil nematode composition showed a significantly strong elevational zonational indicating high degree of ecological specialisation. However, there were cosmpolitan nematode OTUs that were found across all sampled elvations zones. Interestingly, these cosmopolitan nematode OTUs accounted for a greater proportion of the community at high elevations – such that high elevation nematode OTUs had broader elevational ranges on average, providing an example consistent to Rapoport's elevational hypothesis. Furthermore, the relative abundance of feeding guilds also varied across sites, with bacteria-feeding and predatory nematodes accounting for the largest groups at each sampled elavation. Here, we reveal that potential and accessibility of using high-throughput sequencing methods to investigate ecological principles, providing a methods for rapid investigation of patterns without specialized knowledge in taxonomic identification.

COMPARATIVE TRANSCRIPTOMICS OF *STEINERNEMA* AND *CAENORHABDITIS* SINGLE EMBRYOS REVEALS ORTHOLOGOUS GENE EXPRESSION CONVERGENCE DURING LATE EMBRYOGENESIS. **Mortazavi, Ali, M. Macchietto, D. Angdembey, N Heidapour, L. Serra, N El-Ali.** Department of Developmental and Cell Biology, University of California Irvine, Irvine, CA 92697, USA.

Cells express distinct sets of genes in a precise spatio-temporal manner during embryonic development. There is a wealth of information on the deterministic embryonic development of *Caenorhabditis elegans*, but much less is known about embryonic development in nematodes from other taxa, especially at the molecular level. We are interested in insect pathogenic nematodes from the genus *Steinernema* as models of parasitism and symbiosis as well as a satellite model for evolution in comparison to *C. elegans*. To explore gene expression differences across taxa, we sequenced the transcriptomes of single embryos of two *Steinernema* species and two *Caenorhabditis* species at eleven stages during embryonic development and found several interesting features. Our findings show that zygotic transcription initiates at different developmental stages in each species, with the *Steinernema* species initiating transcription earlier than *Caenorhabditis*. We found that ortholog expression conservation during development is highest at the later embryonic stages than at the earlier ones. The surprisingly higher conservation of orthologous gene expression in later embryonic stages strongly suggests a funnel-shaped model of embryonic developmental gene expression divergence in nematodes. Our study provides novel insight into embryonic development across distantly related nematode species and demonstrates that the mechanisms controlling early development are more diverse than previously thought at the transcriptional level.

PATHOGENICITY OF *MELOIDOGYNE INCOGNITA*, *HETERODERA GLYCINES*, AND *ROTYLENCHULUS RENIFORMIS* ON BIRDSFOOT TREFOIL (LOTUS CORNICULATUS) IN ALABAMA. **Moye, Jr., Hayden Hugh[1], N. Xiang[2], K. Lawrence[2], and E. van Santen[3].** [1]Department of Crop, Soil, and Environmental Sciences, Auburn University, Auburn, AL 36849, [2]Department of Entomology and Plant Pathology, Auburn University, Auburn, AL 36849, [3]Agronomy Department, University of Florida, Gainesville, FL 32611.

*Lotus corniculatus* (birdsfoot trefoil) is a common flowering plant in the pea family Fabaceae and native to Eurasia and North Africa used in agriculture as a forage plant and also grown for pasture, hay, and silage due to its non-bloating properties. Auburn University's breeding program for birdsfoot trefoil is attempting to extend the forage's geographic adaptation across the southern United States. Stand decline and galling of the birdsfoot trefoil root systems of the breeding lines was observed at the Plant Breeding Unit of the E.V. Smith Research Center in Tallassee, Alabama in the 2016 season. This location is naturally infested with *Meloidogyne incognita* race 3. A greenhouse study was conducted to determine the pathogenicity and reproductive potential of three nematode species (*Meloidogyne incognita*, *Heterodera glycines*, and *Rotylenchulus reniformis*) common in production areas in Alabama on three varieties (Empire, Norcen, and Pardee) of birdsfoot trefoil. The tests were placed in a RCBD with five replications and the trials were repeated. Data was analyzed using SAS 9.4 PROC GLIMMIX and means separated with Tukey-Kramer's test. All plant varieties (Empire, Norcen, and Pardee) were equally susceptible to *M. incognita* race 3 supporting populations of 117266, 148861, and 143561 per 500 cc of soil, respectively, at 60 days after inoculation. The *M. incognita* eggs per gram of dry root for the three varieties (Empire, Norcen, and Pardee) were 18569, 19890, and 40110, respectively. The reproductive factors (Rf) for *Meloidogyne incognita* race 3 on

the three varieties (Empire, Norcen, and Pardee) were 1.2, 1.5, and 1.4, respectively indicating *L. corniculatus* is a good host for *M. incognita*. The Rf's for *H. glycines* on the three varieties (Empire, Norcen, and Pardee) were 0.0002, 0.0004, and 0.0001, respectively and for *R. reniformis* were 0.01200, 0.02200, and 0.08000, respectively indicating these nematodes did not reproduce on *L. corniculatus*. *Meloidogyne incognita* race 3 was the only nematode species that increased on the three varieties as indicated with Rf, number of eggs, and J2's on birdsfoot trefoil while numbers of *H. glycines* and *R. reniformis* did not increase. The variety Pardee exhibited a 43.9% reduction in fresh biomass and 49.7% reduction in dry biomass compared to varieties Empire and Norcen when infested with *M. incognita* ($P > 0.05$) thus, this nematode could potentially reduce the yield of this birdsfoot trefoil variety. *Meloidogyne incognita* race 3 is an important pathogen of field crops and is wide spread in Alabama and, thus, must be considered in birdsfoot trefoil production systems and breeding programs.

INCIDENCE AND DIVERSITY OF PLANT-PARASITIC NEMATODES ASSOCIATED WITH TURFGRASS IN KOREA. **Mwamula Abraham, Okki [1], Y.J. Kim[1], H.G. Kim[2], and D.W. Lee[1,2].** [1]Department of Ecological Science and [2]School of Ecological Environment and Tourism, Kyungpook National University, Sangju, Gyeongsangbukdo 37224, Korea.

Turfgrass industry is a known economic growth booster worldwide. However, plant-parasitic nematodes are an unacknowledged menace affecting the turfgrass enterprise in many parts of the world. Thus, this study aimed that investigating plant-parasitic nematode diversity within the golf courses in Korea. Eighteen golf courses from 9 provinces were surveyed, and diversity indices including Shannon diversity index (H'), Richness (SR), Evenness (J') and Simpson's dominance ($\lambda$) were used to analyze nematode diversity within the different geographic of turfgrass. Preliminary results remarkably affirmed a diversity of 17 nematode species/taxa belonging to 10 genera and 8 families. *Helicotylenchus pseudorobustus*, *Mesocriconema xenoplax* and *Tylenchorhynchus claytoni* were among the most abundant and prevalent species intercepted in all the surveyed golf courses. The results also revealed significant differences among the different indices across the management zones. The H' and SR values were significantly higher in putting greens than fairway and tee zones. This study highlights the significance of plant-parasitic nematodes in the turfgrass industry of Korea. Moreover, it is the first detailed study of plant-parasitic nematodes associated with turfgrass in Korea

AZADIRACHTIN POWDER FOR CONTROL OF ROOT-KNOT NEMATODES IN TOMATO. **Myers, Roxana, C.L. Mello, and T. Ragasa**. USDA ARS Daniel K. Inouye U.S. Pacific Basin Agricultural Research Center, 64 Nowelo St., Hilo, HI 96720.

Root-knot nematodes cause root galling and yield reductions in many vegetable crops, including tomato. Three organic treatments to improve root growth and reduce nematode infestation were evaluated in a greenhouse potted plant bioassay. Two month old tomato plants in sterilized potting media were treated with either azadirachtin powder, effective microorganisms, a wettable powder containing 2 *Trichoderma* species, or water. In the first experiment, each pot was inoculated with 1000 *Meloidogyne incognita* juveniles and evaluated for nematode penetration by staining the roots with acid fuchsin twenty days later. The number of galls in azadirachtin amended plants was reduced by 59% compared to plants supplemented with *Trichoderma* or water. Roots treated with effective microorganisms were significantly heavier than those in the other treatments. Due to the increased size of the root systems, there was no difference in the number of galls per gram of root when using effective microorganisms compared with the azadirachtin application. In a second experiment to evaluate nematode reproduction, tomato plants were inoculated with 3000 *M. incognita* juveniles and harvested after seven weeks. Azadirachtin powder significantly reduced the nematode population whereas the other treatments had no effect. The population factor was 2.62 when amended with azadirachtin and 5.12, 5.12, and 5.10 when *Trichoderma* spp., effective microorganisms, and water were added. The root mass of azadirachtin treated plants was significantly lighter than the other treatments and resulted in a noticeably different root architecture with longer, finer lateral roots. No differences in plant height were observed. Azadirachtin has potential for lowering root-knot nematode populations in tomato production however yield studies need to be conducted to confirm that the resulting reduction in root size has no negative effect on crop yield.

LESSONS LEARNED DURING THIRTY YEARS OF INFERRING NEMATODE PHYLOGENIES. **Nadler, Steve.** Dept. of Entomology and Nematology, University of California, Davis, CA 95616.

During the last 30 years, the field of nematode phylogenetics has undergone a remarkable transformation. Prior to the application of molecular systematics, most nematode phylogenies were based on one or few morphological features and the individual interpretations of the systematist, and typically lacked formal phylogenetic analysis. Early attempts at nematode molecular phylogenetics mainly focused on protein electrophoresis, methods that were applicable only to relatively closely related species. With the advent of PCR and Sanger sequencing, trees based on universal nuclear ribosomal genes (18S, 28S, ITS) came to dominate nematode phylogenetics, despite known pitfalls of such data (e.g., positional homology inference). Whereas the systematics of other groups of organisms quickly transitioned away from nuclear ribosomal genes to single copy nuclear genes and mitochondrial DNA, nematode phylogenetics instead emphasized adding taxa to 18S ribosomal trees. Even today there is a paucity of well-sampled molecular phylogenies for nematodes that include genes other than 18S rDNA.

One problem resulting from this shortfall is that because gene trees and species trees are not equivalent, it has been difficult to assess if evolutionary patterns in 18S rDNA are supported by analysis of independent loci (concordance principles). More recently, complete mitochondrial genomes of nematodes have provided another locus for independent comparison to 18S trees, and broad outlines of nematode phylogeny are concordant between 18S and mitochondrial genome trees. The expectation of current genomic approaches is that hundreds to thousands of orthologous nuclear loci will be used for nematode phylogenetics, and it is worth considering what advantages and disadvantages to expect from such large multigene approaches. Analyses of phylogenomic datasets for arthropods have shown that not all relationships are reliably resolved, and that the issues affecting data analysis and resolution for these genomic datasets reflect many of the same practical problems and theoretical issues that have been with us for the last 30 years, including the T1/T2 contrast, orthology determination, positional homology inference, and nucleotide compositional bias. Understanding that these potential problems extend to large datasets is essential if the limitations of evolutionary tree inference are to be understood.

USE OF COLOR VEGETATION INDICES FROM UAV AERIAL MAPPING TO EVAUATE NIMITZ® EFFICACY CONTROLING *BELONOLAIMUS LONGICAUDATUS* IN FLORIDA STRAWBERRY. **Navia Gine, P.A.[1], J.W. Noling[2], and C. Rankine[3].** [1]ADAMA Agricultural Solutions Ltd., Raleigh, NC 27604, USA. [2]Citrus Research and Education Center, Institute of Food and Agricultural Sciences, University of Florida, 700 Experiment Station Rd. Lake Alfred, FL 33850. [3]Skymatics Ltd., Suite 100, 1933A 10th Ave SW, Calgary, AB T3C 0K3.

The use of color vegetation indices (CVI) derived from low-altitude aerial photograph orthomosaics obtained using an unmanned aerial vehicle (UAV) were evaluated for the ability to discriminate green leaf area, plant vigor, plant stress, stunting and decay induced by *Belonolaimus longicaudatus* in Florida Strawberry. The two CVIs used in this analysis were the Excess Greeness (ExG) and Excess Redness (ExR), ExG represents the green plant area and ExR indicates the stressed or dead plant area. The vegetation index maps were created at the field scale at different stages of the strawberry growing season. The field was selected for NIMITZ® evaluation based on a long history of high nematode pressure. Programmatically, treatments consisted of one drip irrigation system application of NIMITZ® at 2.9 L/ha, NIMITZ® at 4 L/ha, and Telone® EC at a 112 L/ha in the final stage of the previous year's strawberry crop as a crop termination treatment, including and compared with, an untreated check. In preparation of a fall planting, 7 days prior to strawberry transplanting in September 2016, NIMITZ® was applied at 2.9 L/ha and at 4L/ha, Telone® EC was applied 21 days before transplant at 68.2 L/ha, and again compared with the same untreated controls associated with spring treatments. All treatments were drip applied with 3-hour injection periods except fall Telone® EC applied in 1.5-hours. At transplanting on October 1, 2016, all fall treatments were followed by 14 days of daily overhead irrigation to establish the bareroot transplants. Aerial imaging survey of the experimental area was conducted on December 16th 2016, January 24th and March 22nd 2017 using a DJI Inspire 1 Pro UAS with a DJI Zenmuse Z3 camera and an AerialMediaPros X3 NDVI camera. Image orthomosaics were created using DroneDeploy cloud software platform. Image resolution was high quality, generated at 0.4 inches per pixel. Processed RGB and NDVI maps were analyzed by Skymatics Ltd. Plant row based zonal summary statistic results of each CVI map show significant differences of both NIMITZ® treatments expressing larger canopy size and with more green plant area (ExG) compared to the Telone® EC and Untreated treatments. Conversely, the Telone® EC treatment showed numerically more stressed, reduced canopy and dead areas (ExR) than within NIMITZ® treated areas, the untreated control showed significantly greater ExR areas compared to either NIMITZ® treatments. This work suggests that the use of CVIs from unmanned aerial imaging can be useful to rapidly assess nematode damage and nematicide treatment efficacy in a very practical, non-invasive, and quantitative way, with implications for use in related plant pathology studies.

NEMATODES AS INDICATORS OF CHANGES IN AGRICULTURAL MANAGEMENT **Ney, Laura, D.F. Franklin, K. Mahmud, A. Hattabaugh.** UGA Dept. of Crop and Soil Sciences, Sustainable Agriculture Lab, 3111 Miller Plant Sciences Bldg. Athens, GA 30602.

Nematodes, often associated with yield loss and plant disease by agriculturists, have been recognized as useful soil health indicators by ecologists for decades. Plant parasitic nematodes (PPN) make up only a fraction of the nematode community living in soils. Spanning across nearly every trophic group, nematodes occupy key positions as primary and intermediate consumers in the soil food web and can therefore be used as a measures for soil health and productivity related with their food resources. We looked at nematode community structure at a trophic level as one component of measuring the effect of the novel microbial inoculant LEM (Local Effective Microbes) on soil health and productivity. Two years of data collected on soybean (*Glycine max*) plots receiving a composted broiler litter amendment, show an increase in bacterial-feeding and fungal-feeding nematodes among LEM-treated plots after two years of LEM application. While higher numbers of PPN were found in LEM plots compared to controls, the relative proportion of PPN to the total nematode community was not significantly greater. By the end of the second year, LEM plots ranked higher on the Maturity Index, Structural Index and Shannon's Diversity Index than the controls. As a part of this research, we also compared the maturity and diversity of nematode communities between soils under two years of forage production receiving swine effluent amendments versus soils under soy production receiving composted broiler litter amendments. While many of the indices developed by ecologists use

nematodes to monitor the recuperation of non-agricultural soils after a disturbance, these indices could also be useful tool for measuring the health and resilience of agroecosystems. This research will provide valuable information on how nematode communities respond to different agricultural management systems and specifically how they respond to the application of LEM.

IN-FIELD EVALUATIONS OF NEMATICIDAL COMPOUNDS USING SIMULATED DRIP IRRIGATION APPROACHES FOR CONTROL OF THE STING NEMATODE, *BELONOLAIMUS LONGICAUDATUS*. **Noling, Joseph**. University of Florida, IFAS, Citrus Research & Education Center, Lake Alfred, FL 33850.

Drip irrigation delivery of nematicidal compounds is extensively used in Florida to manage plant parasitic nematodes. Complicated delivery manifolds are constructed to direct irrigation flow as nematicidal compounds are metered into the irrigation system to distribute toxic concentrations to specific plots over long injection periods to maximize soil coverage. A simpler approach, simulating drip irrigation delivery, was evaluated in Sting nematode infested fields during 2017 using perforated plastic irrigation spikes threaded to the top of 0.6 liter water bottles. To conduct a field evaluation, concentration gradients of different nematicidal compounds were created within replicated water bottles, the spike delivery stake installed and the bottle inverted and pressed 12.7 cm into soil within the strawberry plant root zone. Soil samples were then procured from the root zone of each treated plant 7 to 14 days after application to assess Sting nematode soil population density and compared with untreated plants in the adjacent row of plants on the same raised plant bed. Changes in plant growth were also recorded before and after treatment application. Preliminary tests utilizing a water soluble, blue soil staining dye confirmed the desired vertical and horizontal spread of the water front outward from the single drip emission point on the spiked stake. Subsequent studies with Majestene®, Nimitz® (Fluensulfone), and metam potassium (Kpam®) have been successfully conducted to quantify nematicidal efficacy and crop phytotoxicity of the different compounds. Majestene provided no apparent benefit either in providing Sting nematode control or improvement to plant growth at any of the concentrations tested. Metam potassium proved effective for Sting nematode control at concentrations in excess of 500 ppm, and for crop termination / plant kill at concentrations in excess of 2000 ppm. Fluensulfone concentrations required to effectively control Sting nematode were substantially higher in these field trials than in other studies which report nematicidal activity with other plant parasitic nematodes. These results from the fluensulfone study indicate possible species effects or that a time-dependent dose-response curve, describing the progressive increase in exposure leading to death needs to be accounted for in a time series sampling program. The new approach simulating drip irrigation delivery of a nematicide has proved to be a very quick and easy system of testing both old and a plethora of new compounds claiming nematicidal activity. This work also suggests the need for additional, more defining research to quantify the dose response relationship for different nematode species, optimal concentration and injection volume, and to clarify appropriate times within the cropping season in which efficacy and plant growth benefit to infected plants can be effectively achieved. The ability to utilize commercial fields with existing nematode problems is value added in terms of satisfying starting conditions of nematode pressure, monitoring potential crop recovery and for involving grower participation and observation within field research.

ACHIEVING STING NEMATODE CONTROL IN FLORIDA STRAWBERRY USING VERTICAL MANAGEMENT ZONES. **Noling, Joseph[1], G.E. Vallad[2] and N. Boyd[2].** [1]University of Florida, IFAS, Citrus Research & Education Center, Lake Alfred, FL 33850, [2]Gulf Coast Research and Education Center, Wimauma, FL 33598.

During 2017, field studies were conducted to demonstrate the importance of deep fumigant placement and management of Sting nematode, *Belonolaimus longicaudatus*, as a composite of vertical management zones located above and below a gas impermeable traffic pan. To target deep soil profiles, new fumigant application systems were developed to shank inject fumigants 40 cm deep. The fumigant treatments included deep shank applications of 1,3-dichloropropene (Telone II™; 18.4 L/ha) with or without Telone C35 (46 L/ha), PicClor60 (38 L/ha), PicClor80 (35.2 L/ha), and Pic100 (33 L/ha) applied in-the-bed. The differing formulations allowed evaluation of increasing chloropicrin use rates from 21.4 to 45.9 kg/ha to effectively manage Sting nematode and Charcoal Rot, caused by *Macrophomina phaseolina*. Other fumigant treatments evaluated included bed shank and drip treatments of Paladin® Pic (79/21%)(46 L/ha), drip applied metam potassium (KPAM®; 95 L/ha) and allyl isothiocyanate (Dominus® 46 L/ha). An untreated control with and without the deep shank Telone II ™ (18.4 L/ha) treatment was included for comparison. Strawberry yields were unresponsive to application rates of chloropicrin greater than 21.4 kg/ha. This was partially attributed to the reduced level of Sting nematode control with increasing rates of chloropicrin application to the plant bed (vertical management zone 1). Strawberry yields were significantly ($P<0.001$) increased for each Telone Chloropicrin formulation applied to the plant bed when Telone II was deep shank applied below the traffic pan within vertical management zone 2. A reduced level of response for Telone C35 with deep shank Telone II was observed and is thought to be attributed to repeated treatments to the same beds for two consecutive years of strawberry production. In other grower trials where deep shank treatments have been repeatedly applied for nematode control, yields were less responsive with each annual application suggesting an every other year application may only be required to manage nematodes in deep soil profiles to minimize strawberry crop losses. Strawberry yields increased 20% above that of the untreated control following only the deep shank Telone II treatment (18.4 L/ha). Additional yield increases of 32 to 38

percent were observed when the deep shank treatment was supplemented with an in-the-bed fumigant treatment. Additional chloropicrin in itself did not reduce disease induced plant mortality at seasons end, but deep shanking TeloneII significantly reduced final harvest Sting nematode populations compared to the untreated controls or in-the-bed only fumigant treatments. KPAM® and Dominus® produced strawberry yields equivalent to that of the untreated control. Use of deep shank Telone II (18.4 L/ha) has largely resolved Sting Nematode induced yield losses in FL strawberry. We believe a primary cause of inconsistent nematode control using methyl bromide alternatives has been identified, and that supplement fumigant applications, which consider the importance of vertical management zones, will be required to manage nematode pests in Florida strawberry.

A FIELD SCALE OF PLANT-PARASITIC NEMATODES AND CROP GROWTH AFTER NEEM CAKE AMENDMENT IN THE BRAZILIAN SEMIARID. **Oliveira, Ana Karina dos Santos, D.A.H.S. Leitão, D.B. Castro, E.M.R. Pedrosa.** Agricultural Engineering Dept., Federal Rural University of Pernambuco, Recife, PE, Brazil 52171-900.

Neem cake amendment has been successful used as a management strategy for plant-parasitic nematode integrated control. We hypothesized plant-parasitic nematode densities is lower in neem-cake-treated areas, in contrast to the free-living nematodes, especially bacterivores, increasing organic matter benefits on green pepper crop. A field study was carried out from April to June 2013 in a family-farming plot in the semiarid region of Pernambuco state, Brazil. Approximately 20,000 green pepper seedlings were transplanted into 50 cultivation rows prior to the experiment. The plot was divided into two smaller areas (A and B) of 2,304 $m^2$ each. A regular 49-point regular grid was delimited in each area for soil sampling, with 8 m spacing between points. Soil samples were collected twice: 1) 7 days after transplanting (before neem cake amendment) and 2) at harvest, 68 days after transplanting (47 days after neem cake amendment). Plant height and stalk diameter were measured at harvest. Soil in A was amended 21 days after transplanting by incorporating 100 g of neem cake per linear meter of cultivation row; no other additional management was performed in B. Nematodes were classified according to feeding habits into five trophic groups (bacterivores, fungivores, omnivores, predators and plant parasites). Plant-parasitic nematodes were further identified at genus or family level. Nematode data was subjected to analysis of covariance in order to verify the changes in nematode population densities before and after neem cake amendment. Plant growth variables means were compared through Tukey's test at 5% of probability. Plant height and stalk diameter were greater in A, indicating that the incorporation of neem cake to the soil in the semiarid was useful to increase crop productivity. The abundance and dominance of plant-parasitic nematodes were greater at the beginning of the experiment due to ruderal plant species present in both areas. After neem cake amendment, bacterivorous nematodes were the most dominant trophic group, indicating a highly productive system, with rapid nutrient cycling, reinforcing the organic matter benefits. Criconematids were favored by the greater plant growth in A, while *Trichodorus* and *Paratrichodorus* densities were significantly suppressed by neem cake. Bacterivorous, free-living and total nematode densities were significantly higher after neem cake amendment.

*DITYLENCHUS GALLAEFORMANS:* A POTENTIAL BIOLOGICAL CONTROL AGENT FOR INVASIVE PLANT *CLIDEMIA HIRTA*. **Oliveira, Samara Azevedo[1]; H., Boatwright,[1] P.M., Agudelo,[1] and S.J., DeWalt.[2]** [1]Plant and Environmental Sciences Dept., Clemson University, [2] Biological Sciences Dept. Clemson University, Clemson, SC 29634.

*Clidemia hirta* is a shrub native to Central and South America. This plant has become an invasive species in regions of Africa, Asia, Australia and islands in the Pacific and Indian Oceans. This invasive species competes with native Hawaiian rainforest plants and is therefore a target for control. Several management techniques have been applied against *C. hirta* in Hawaii, but none of the activities have been successful. Oliveira et al. (2003) described a new species of nematode, *Ditylenchus gallaeformans,* found causing severe leaf galling symptoms in *Miconia* sp. in Minas Gerais, Brazil. With a general objective to reduce the dissemination of *C. hirta* in Hawaiian rainforests, this study tests the potential of *D. gallaeformans* as a biological control agent against *C. hirta*. Specifically, we aim to assess whether pathogenicity and virulence of the nematode depends on the host species, host genotype, and geographic source. In this phase of the experiment, we worked with plants and nematodes from Costa Rica and Trinidad and with the invasive genotype of *C. hirta* from Hawaii. Plants from the two native and one invasive location were cultivated in a greenhouse at Clemson University. *D. gallaeformans* was obtained from symptomatic Melastomataceae leaves collected in Costa Rica and Trinidad. The identity of the nematode species was confirmed by DNA barcode (ITS-1). In the first part of the experiment, we inoculated plants from Costa Rica and Hawaii with nematodes isolated from different melastomes from Costa Rica. In the second part, plants from Trinidad and Hawaii were inoculated with *D. gallaeformans* collected from Trinidad. The Hawaiian plants inoculated with nematodes from Costa Rica showed symptoms at 60 days after inoculation. After 5 months, the symptoms did not progress. Plants from Costa Rica inoculated with nematodes from the same location did not show any symptoms. In the second part of the experiment, plants from Hawaii and Trinidad were inoculated with nematodes from Trinidad. Plants from Hawaii developed galls starting two weeks after the inoculation, while those from Trinidad took three weeks to show symptoms. The galls on Hawaiian plants were more pronounced than on Trinidad plants. Thus, in both experiments, the nematodes from Trinidad caused more symptoms in plants from Hawaii than in the plants from the same origin as the nematode. We continue to test the susceptibility of different plant genotypes to nematodes from different origins with the goal of finding the most effective nematode population to control *C. hirta* in Hawaiian rain forests.

SOYBEAN CULTIVARS AND FUMIGATION AGAINST *ROTYLENCHULUS RENIFORMIS* IN A COMMERCE SILT LOAM SOIL. **Overstreet, Charles[1], E.C. McGawley[1], D.M. Xavier-Mis[1], M. Kularathna[1], and D. Burns[2].** LSU AgCenter; [1]Dept. of Plant Pathology and Crop Physiology, Baton Rouge, LA 70803, [2]Tensas Parish, St. Joseph, LA 71366.

Reniform nematode (*Rotylenchulus reniformis*) is highly variable in reproduction and pathogenicity on soybean cultivars. The objectives of this research were to evaluate cultivars with various levels of resistance to this nematode and the impact of fumigation in a field with a Commerce silt loam soil previously planted with cotton. A field study was conducted from 2014-2016 with nine cultivars (three moderately resistant and six susceptible to reniform nematode) each year and two nematicide treatments (the fumigant 1,3-dichloropropene and a no-nematicide control) with six replications. Soil samples were collected at-planting and after harvest for nematode analysis. The fumigant significantly decreased populations of the nematode at the time of planting an average of 87% and after harvest by 59%. There were also significant differences with reniform nematode population density after harvest among the cultivars with the moderately resistant cultivars generally having the lowest populations. The fumigant did not result in significant yield increases in any year. Although there were significant differences in yield among the cultivars each year, there was no fumigant by cultivar interaction. Cultivars in 2014 and 2015 that were moderately resistance to reniform nematode provided a significant increase of 269 and 322.8 kg/ha, respectively over cultivars that were susceptible. These studies indicate that under the soil and environmental conditions present in a Commerce silt loam field, cultivar selection was more important than the use of a nematicide to manage reniform nematode.

PARASITIC NEMATODES SPREAD BY INVASIVE COCKROACHES. **Ozawa, Sota[1], A. Takuya[2], H. Koichi[1].** [1]Department of Environmental Biology, College of Bioscience & Biotechnology, Chubu University. 1200 Matsumoto, Kasugai, Aichi, 487-8501 Japan, [2]Tohoku Research Center, Forestry and Forest Products Research Institute. Morioka, Iwate, 020-0123 Japan.

Cockroaches, referred to as ''living fossils'', have about a 350-million-year history and constitute one of the most diverse groups of insects on earth with around 4,500 species that inhabit diverse environments. The American cockroach *Periplaneta americana*, the smokybrown cockroach *P. fuliginosa*, and the German cockroach *Blattella germanica* are considered to be serious urban sanitary pests spread by modern human economic activities. Thelastomatid parasitic nematodes (Spirurina, Clade III) are obligate parasites of Blattodea insects' hindguts. Previously we showed that the nematode *Leidynema appendiculatum*, a parasite of *P. fuliginosa*, is capable of colonizing a broad range of hosts. This nematode could infect five cockroach species belonging to three families in two suborders by experimental inoculation. We hypothesize that a consequence of the global dissemination of parasitized cockroaches will be the infection of native cockroach species with parasitic nematodes. *P. japonica* is a Japanese domestic cockroach found primarily in northern Japan, was thought to be originally infected with the parasitic nematode *Protrellus* species. *P. fuliginosa*, found mainly in central and western Japan, is an invasive species thought to have disseminated from East China. To date, all *P. fuliginosa* collected from outdoor and indoor locations in Japan were found to be infected with *L. appendiculatum*. To monitor dissemination of *L. appendiculatum* we collected *P. fuliginosa* and *P. japonica* from three regions: (1) urban green spaces of Minato city (Tokyo, Japan) where *P. fuliginosa* and *P. japonica* co-inhabit; (2) urban green spaces of Minato city (Tokyo, Japan) where only *P. fuliginosa* is found; and (3) secondary-growth forests (Japanese cedar forest, Oak forest) of Iwate Prefecture (Iwate, Japan) where only *P. japonica* is found. We found that *P. japonica* captured in urban spaces but not secondary-growth forests, is colonized by *L. appendiculatum*. Our results are consistent with the hypothesis that *L. appendiculatum* is a general parasite of cockroach species, and is currently expanding its global range via world-spreading cockroach vectors such as *P. fuliginosa*.

RNAI SILENCING OF VARIOUS FUNCTIONAL GENES OF *HETERODERA AVENAE*. **Papolu, Pradeep, D. Singh, Vikas and U. Rao.** Division of Nematology, ICAR- Indian Agricultural Research Institute, New Delhi-110012, INDIA.

The cereal cyst nematode (CCN), *Heterodera avenae*, is one of the yield limiting constraints in wheat and barley around the world. To gain insight into its repertoire of parasitism genes, comparative genomic analysis was done using the information available for different nematodes and identified 40 pioneer genes in *H. avenae*. The selected genes were classified in to four major groups *viz*., neuropeptides, proteases, cell wall degrading enzymes and secretary proteins. In the present study, all these genes were cloned from cDNA of J2s and combinatorial *in vitro* RNAi strategy was used for simultaneous knock down of any two selected genes in a single soaking solution to determine the additive or synergistic effect on nematode infection and reproduction in order to establish the long term effects of gene silencing. Freshly germinated wheat seedlings were infected with CCN juveniles soaked in dsRNA of selected gene combinations and observations were recorded on the number of cysts produced at crop maturity. The results revealed that combinatorial RNAi silencing of two genes (RAN-BPM and VAP) was found to be the most effective in reducing the number of cysts by 93%, eggs per cyst by 44% and multiplication factor by 54% compared to the control. In other three gene combinations *viz*., RAN-BPM, CBR chitinase and CBN chitinase, we observed reduction in cysts by 83 %, eggs per cyst by 9 % and multiplication factor by 84 % over control. The infection results are supported by alteration in the transcript expression of target genes as quantified by qRT-PCR. However, we didn't find any significant additive or synergistic effect of combinatorial RNAi on the nematode infection and reproduction when compared to single gene silencing.

DISCERNING FUNCTIONAL MOLECULAR PATHWAYS UNDERLYING *MELOIDOGYNE INCOGNITA* - *PASTEURIA PENETRANS* INTERACTION USING TRANSCRIPTOMIC APPROACH**. Phani, Victor[1], K.G. Davies[2] and U. Rao[1].** [1]Division of Nematology, ICAR-Indian Agricultural Research Institute, New Delhi- 110 012, India. [2]School of Life and Medical Sciences, University of Hertfordshire, Hatfield, Herts- AL10 9AB, UK.

A conceivable situation currently exists in modern farming where plant-parasitic nematodes would have a major impact on global food production system. Amongst the major emerging gridlocks, the root-knot nematode (RKN) *Meloidogyne incognita* is a widely adapted endoparasitic species attacking majority of crops inflicting serious yield and quality losses. Difficulty in implementing single serviceable management tool and global restriction on traditional nematicides has put forward the necessity of finding alternative to sway out this ''hidden enemy''. *Pasteuria penetrans*, a Gram-positive, endospore forming soil bacteria is a potent candidate in this run to manage the RKN females by turning it into a bacterial sac and thereby useful as a bio control agent. The attachment of endospores to the cuticle of second stage juveniles of RKN is the primary step of infection. But the activation of spores depend strongly on combination of host and parasite genotypes and very little is known about this interaction at molecular level. Hence RNA-Seq was employed to detect the differential expression profile between encumbered and non-encumbered juveniles of RKN. The de-novo sequence assembly using Trinity Assembler resulted in 68,417 final transcripts at N50 of 1053 bp of which 57% were annotated by blastx. A total of 759 genes were differentially expressed including 314 up- and 445 down-regulation. About 34 membrane associated proteins were detected with down regulated oxido-reduction, glycosylation, ubiquitination, transmembrane signaling and neuropeptide signaling pathways and up regulated metal ion transport, hydrolase and endonuclease activity. Fatty acid and retinol binding protein, zinc finger protein, heat shock protein, cytochrome oxidase and chorismate mutase were found to be significantly down regulated indicating host defense suppression supporting bacterial multiplication. Further, up regulation of PapD like protein, Selenium binding protein and protein kinases indicate their probable involvement to arrest the infection process from progress. Interestingly, up-regulation of transcripts coding for major sperm proteins in nematodes was also detected in such an early developmental stage indicating reproductive interference. The transcripts exhibited high similarity to animal parasitic nematodes *viz*., *Ascaris suum*, *Strongyloides ratti*, *Loa loa*, *Necator americanus*, *Ancylostoma duodenale*, *Haemonchus contortus* and *Wuchereria bancrofti* which indicates higher orthology with parasitic species. Plant parasitic species include *Meloidogyne hapla*, *M. arenaria*, *Aphelenchoides besseyi* and *Bursaphelenchus xylophilus* in this list. Work is in progress to functionally validate some of the differentially expressed genes by RNAi and qPCR. This study provides a comprehensive insight of the RKN-*Pasteuria* interaction at molecular level to understand a naturally co-evolved pathogen-hyperparasite intercommunication system.

NEMATODES AS A LEARNING TOOL FOR UNDERSTANDING BIODVERSITY AND THE POTENTIAL USE OF PHORETIC INSECT AND SOIL DWELLING NEMATODES AS FORENSIC POSTMORTEM INTERVAL INDICATORS. **Phillips, Gary, L.S. Taylor, S.K. Pothula, and E.C. Bernard.** University of Tennessee, 2505 E. J. Chapman Drive, 370 Plant Biotechnology Building, Knoxville, TN 37996-4560 USA.

During four years of research, 60,000+ nematodes were dissected from the gastrointestinal (GI) tract of 900 millipedes spanning 47 species in Rhigonematida and Oxyuridomorpha. *Coronostoma* spp., *Stauratostoma shelleyi*, *Thelastoma* spp., and *Heth pivari* are some of the 20+ new taxa that have been discovered in temperate North American millipedes. Millipedes greater than 2 mm typically harbor nematodes, almost all of which appear to be harmless kleptoparasites. Some nematodes are geographically restricted; for instance, *S. shelleyi* is known only from the Blue Ridge-Smokies area of the mid-South. Intensive sampling has revealed high diversity among some taxa formerly known only from the tropics, such as *Coronostoma*. Four temperate-zone North American species of this genus have been collected as opposed to six species from all tropical regions. With more than 12,000 species of millipedes, countless more nematode taxa await discovery. The biodiversity, abundance and prevalence of nematodes inhabiting the GI tract of millipedes can serve as a tool for teaching about host-parasite interactions. By dissecting the millipede intestine, students can observe interactions between the host, nematodes, protists, fungi, bacteria, and other organisms. This approach to biodiversity has the potential to be cost-effective, present a unique perspective on host-parasite interactions, foster creativity, and sharpen observational skills. In another project, we have hypothesized that soil-dwelling and insect-phoretic nematodes can be used in conjunction with insect data to help estimate forensic postmortem interval. Similar to flies and beetles, nematodes have a successional pattern and changes in abundance and diversity occur during each stage of vertebrate decomposition. In a current study, six North American beaver cadavers were placed separately into predator-proof cages in an oak forest in late March, 2017. Probes were placed inside each beaver and internal temperatures were recorded, along with ambient and soil temperatures. Six control plots without carcasses were established two meters from each cage. Soil samples were taken from cage and control plots prior to placement of the beaver, during each stage of decomposition, and at the interface layer between carcass and soil. Diptera and Coleoptera arriving at the cadavers were collected and examined for phoretic nematodes, then placed in Petri dishes of NGM agar + *E. coli* OP50 to obtain phoretic or internal nematodes. Nearly all collected beetles, especially Silphidae, were found to carry *Rhabditoides inermis* under the elytra, but internal nematodes were not observed following dissection. No flies were found to be transporting nematodes. Nematode community composition was similar in cage and control plots until five days

after placement, when proportions of non-soil Rhabditidae, especially *Pelodera* and *Rhabditella* spp., began increasing rapidly in relation to other nematodes. These community composition changes appear to have predictable trajectories that could permit more precise estimation of time of death.

INFLUENCE OF HUMAN-INDUCED DISTURBANCE ON NEMATODE RICHNESS AND ABUNDANCE: A META-ANALYSIS. **Pothula, Satyendra, K.[1], P.S. Grewal[4], R.M. Auge[2], A.M. Saxton[3], E.C. Bernard[1].** [1]Department of Entomology and Plant Pathology, [2]Department of Plant Sciences, [3]Animal Science, University of Tennessee, Knoxville TN 37996 USA, [4]School of Earth, Environmental, and Marine Sciences, University of Texas Rio Grande Valley, 1201 West University Drive, Edinburg, Texas 78539-2999 USA.

Nematodes are at a central place in the soil food web. The structure of nematode communities provides useful information on the condition of the soil food web, since nematodes have different levels of sensitivity to disturbances and specificity to food sources. Our objective was to determine the effect of human activity on the richness and abundance of nematodes found in different ecosystems. Meta-analysis was conducted using comprehensive meta-analysis software to compare the overall richness, overall abundance, richness and abundance of each trophic and colonizer-persister (CP) group among urban, agriculture, disturbed grassland, natural grassland and forest ecosystems. Richness and abundance of nematodes per 100 g and per 100 $cm^3$ of soil were separately analyzed. A total of 598 relevant articles were found by using a sequence of different search terms, out of which data were extracted from 111 articles that met the inclusion criteria. Results per 100 g soil basis indicate that the overall genus-level richness of nematodes was highest in relatively undisturbed ecosystems such as forest (44 genera) and natural grassland (31) compared with more disturbed ecosystems such as disturbed grassland (29), urban (28), and agriculture (25). The richness of nematodes of all trophic groups and CP classes except CP-1 was highest in forest ecosystems. In contrast, overall nematode abundance was highest in disturbed grassland ecosystems (1426/100 g soil) followed by forest (1400) and agriculture (1320). The abundance of lower CP class (CP-1, CP-2, and CP-3) and bacterivore fungivore and herbivore trophic group nematodes was highest in disturbed ecosystems. The abundance of CP-4 and CP-5 classes, which include omnivore and predator trophic groups, was highest in undisturbed ecosystems. An ecosystem with weak or no perturbation supports higher richness, which enhances the complexity of soil food webs. On the other hand, an ecosystem with human manipulations, especially the addition of organic and inorganic nutrients that enhance microbial activity, increase nematode abundance but not richness. Therefore, agricultural intensification and urbanization apparently negatively impact nematode community richness, which is critical for the maintenance of soil ecosystem services and resilience.

EVALUATION OF *MELOIDOGYNE FLORIDENSIS* AND *M. ARENARIA* ON RESISTANT PEACH ROOTSTOCK CV. FLORDAGUARD. **Qiu, Sai[1], J. Chaparro[2], T. Beckman[3], J.A. Brito[4], and D.W. Dickson[1].** [1]Entomology and Nematology Department, [2]Horticultural Sciences Department, University of Florida, Gainesville, FL 32611, [3]USDA ARS, Byron, GA, [4]Divison of Plant Industry, Gainesville, FL 32614.

*Meloidogyne floridensis*, the peach root-knot nematode, is only reported to occur in Florida and is considered as a potential threat to the newly developing peach industry. In addition to *M. floridensis*, *M. arenaria* has recently been reported infecting peach in Florida. The objectives of this project were to determine whether *M. floridensis* and *M. arenaria* infect the root-knot nematode resistant peach rootstock cv. Flordaguard; to determine the host race of these nematode species, if any; and to determine the host status of peach rootstock cvs. Flordaguard, Flordaglo, Lovell, and Okinawa to these two nematode species. Of four isolates of *M. floridensis* tested, only one that was recently extracted from Flordaguard consistently infected this rootstock in greenhouse experiments. Two isolates of *M. arenaria* collected from commercial peach orchards both infected Flordaguard. This is the first report of *M. arenaria* infecting Flordaguard peach in Florida. Based on differential host tests, both *M. arenaria* species were designated as race 3. There was no infection of peach rootstock cv. Okinawa by *M. arenaria*.

COMPETING GENE-DOSAGE IN THE REGULATION AND EVOLUTION OF THE *PRISTIONCHUS* FEEDING-STRUCTURE POLYPHENISM. **Ragsdale, Erik J., N.A. Ivers, and L.T. Bui.** Department of Biology, Indiana University, Bloomington, IN 47405.

Nematode diversity is especially conspicuous in the wide array of feeding strategies different species are able to assume. Even within species, some nematodes have the ability to fill starkly different ecological roles according to resource availability. Polyphenism, a feature of multiple nematode groups, is the ability for development to switch between alternative outcomes in response to environmental cues. Although the genetic basis for morphological polyphenism in general was until recently poorly understood, a nematode model system, *Pristionchus pacificus*, has enabled studies of the developmental genetic interpretation of environmental cues. In this species, like several other diplogastrid nematodes, adult feeding-structures assume one of two different morphologies, one of which has more or larger teeth and confers the ability to prey upon other nematodes. Previously, the stomatal polyphenism was found to be regulated by a novel sulfatase (EUD-1), which indirectly inactivates a nuclear hormone receptor (NHR-40). By analyzing other genetic mutants that deactivate the

polyphenism switch, we discovered that another enzyme, a novel sulfotransferase that we have named SEUD-1 (suppressor-of-*eud-1*), also takes part in the developmental switch. By manipulating gene dosages of *eud-1* and *seud-1* by crosses and transgenic experiments, we found that the relative numbers of functional alleles of the two genes determined the ratios of feeding-morphs among nematodes. Thus, the putative signal-modifying enzymes EUD-1 and SEUD-1 compete for the outcome of the switch, offering a two-part, rheostat-like mechanism for dialing up or down the likelihood of a developmental decision. We also tested whether relative gene-dosage may be responsible for different polyphenism phenotypes among species. Specifically, we performed crosses of *P. pacificus* to another, reproductively compatible *Pristionchus* species in which *seud-1* was recently duplicated. By manipulating gene dosage with interspecific hybrids, we found that gene duplication can amplify the effects of a polyphenism switch gene on the phenotype. Together, our results suggest that the response of development to the environment, particularly one that produces disparate adult morphologies, can evolve directly by amplifying those genes. In summary, our study provides the first genetic insight into how the molecular regulation of polyphenism evolves to produce new, developmentally conditioned phenotypes, particularly in a trait that is a hallmark of nematode diversity.

UNDERSTANDING THE BIOLOGY AND ECOLOGY OF NATURAL FUNGAL ANTAGONISTS OF SOYBEAN CYST NEMATODES FOR BIOLOGICAL CONTROL. **Rajendran, Deepak[1], E.K. Bushley[2], S. Chen[1].** [1]University of Minnesota, Department of Plant Pathology, 1991 Upper Buford Circle, St. Paul, MN 55108, USA. [2]University of Minnesota, Department of Plant and Microbial Biology, 1479 Gortner Avenue, St. Paul, MN 55108, USA.

A good biological control agent must not only affect the pathogen *in vitro*, but must also establish, survive, and thrive in the environment where it needs to have effective biocontrol. Rather than using foreign organisms that could either fail to establish and engage the pathogen in native environments or outcompete native microbial communities, using local, naturally occurring antagonists as biocontrol agents seems prudent. The present study used both morphological characteristics and ITS sequence for identifying the mycobiome in the soybean cyst nematode (SCN) cysts from a long-term soybean-corn crop rotation experiment. Thus far, research on SCN cyst mycobiome has mostly been based on morphology alone. In this study, fungi were isolated from approximately 5000 cysts collected over three consecutive years across two seasonal timepoints—in midseason and at harvest. Fungal isolates were clustered together using their ITS sequences with the help of algorithms provided by Minnesota Supercomputing Institute, searching against both Unite and NCBI databases. This is the first study where both morphology and molecular analysis are being combined to identify fungal isolates associated with SCN. The main genera found were primarily in the order Hypocreales, including Nectriaceae *Fusarium, Dactylonectria, Cylindrocarpon, Clonostachys,* and *Mariannaea* as well the Chaetothyriomycete *Exophiala* and the Dothidiomycete *Leptosphaeria*. Different crop rotation sequences had no significant effect on the fungal community composition associated with cysts. The isolates were then tested for their parasitism on SCN cysts and eggs, and for their ability to prevent egg hatch. Egg parasitism was scored using the egg parasitic index (EPI) on a scale of 1 to 10 based on the percentage of eggs colonised. Several *Fusarium* spp. exhibited high EPI and hence are good candidates of biocontrol of SCN. Eggs were hatched in tissue-culture wells free supernatant of these fungi and several isolates also inhibited juvenile hatching along with being egg parasites. Once fungal isolates that exhibit high toxicity are identified, they will be further examined to identify specific compounds involved. Future directions also include ecological studies investigating the ability of these fungi to colonize different soil types and establish without fungistasis, and testing candidate biocontrol agents in greenhouse trials. Since soil has microbial communities rather than single microbe colonizing the entirety, we will also use combinations of fungal isolates as per their abundances detected during their initial isolation and identification to evaluate biocontrol potential.

EXPLORING OVERLAP BETWEEN LATERAL ROOT ORGANOGENESIS AND RENIFORM NEMATODE FEEDING SITE FORMATION IN SOYBEAN. **Redding, Nathan[1], P. Agudelo[1], and C.E. Wells[2].** [1]Department of Plant and Environmental Sciences and [2]Department of Biological Sciences, Clemson University, Clemson, South Carolina, USA 29634.

Reniform nematode (*Rotylenchulus reniformis*) is a soil-borne plant parasite with a broad host range and wide global distribution in tropical, subtropical, and warm temperate areas. To establish infection, a female nematode forcibly penetrates the root epidermis and cortex. Upon reaching the endodermis, the nematode creates a permanent feeding site called a syncytium, which is defined by an amalgamation of individual roots cells that form a dense cytoplasmic network. To elucidate the molecular basis behind feeding site formation, we performed an RNAseq study using a soybean (*Glycine max*) split-root system. Inoculated and control root samples were taken from three split-root replicates at four points across a twelve-day time course, resulting in a total of 24 samples from 12 plants. Gene expression abundance and differential expression analysis revealed that over 6,000 genes were differentially expressed on at least one sampling date (FDR = 0.01, $|\log2FC| \geq 1$). Following annotation with enzyme code, KEGG pathway, and GO terms, enrichment analysis revealed 507 gene sets to be significantly enriched or depleted in inoculated roots (FDR = 0.05). Several differentially expressed genes were associated with enriched GO terms involving auxin-based processes. These included components of auxin response modules (ARFs and IAAs), auxin transporters (PINs), and signal regulators (CLEs). Of primary interest were many genes

whose homologs play a role during lateral root formation in *Arabidopsis*. To examine nematode effects on root architecture, imaging analysis was performed on split and intact root systems. Correlations were made between differential gene expression and root architecture, indicating potential overlap between plant-directed lateral root formation and nematode-induced organogenesis.

IMPACT OF ROOT KNOT NEMATODES AND FUSARIUM WILT ON TOMATO ISOLINES THAT HAVE OR LACK RESISTANCE TO THESE PESTS. **Regmi, Homan[1], C. Land[2], G.E. Vallad[2], S.F. Hutton[3], and J. Desaeger[1]**. [1]Entomology and Nematology Department, University of Florida, Gulf Coast Research and Education Center (GCREC), 33598, [2]Plant Pathology Department, University of Florida, GCREC, 33598, [3]Horticultural Sciences Department, University of Florida, GCREC, 33598.

Tomato cultivars with *Mi* gene offer good protection against the most common root-knot nematode species in Florida (*Meloidogyne incognita, M. javanica* and *M. arenaria*). However, *Mi*-tomatoes are not commonly used by growers in Florida, due to the limited availability of acceptable varieties with *Mi*, the heat sensitivity of *Mi*, and mostly because of reliance on soil fumigation to control other soilborne pests and diseases than root-knot nematodes. One of the major tomato diseases in Florida is *Fusarium* wilt caused by the fungus *Fusarium oxysporum* f. sp. *lycopersici* (Fol). To increase adoption of *Mi* tomatoes in Florida (and to reduce the dependency on soil fumigation), acceptable tomato cv's are needed that have at least resistance to both these pathogens. The impact of root-knot nematode (*M. javanica*, Mj) and Fol was therefore evaluated on four isolines of the popular cultivar Tasti-Lee® (TL) with or without *I-3* and/or *Mi* genes. During spring of 2017, a greenhouse experiment was set-up at the University of Florida's Gulf Coast Research and Education Center (GCREC) to evaluate original TL ($I\text{-}3^+$, $Mi^-$) and its 3 hybrid isolines, TL ($I\text{-}3^+$, $Mi^+$), TL ($I\text{-}3^-$, $Mi^+$) and TL ($I\text{-}3^-$, $Mi^-$). The isolines were subjected to three different nematode levels (high, low and none) and two Fol inoculum levels (inoculated and non-inoculated). Tomato plants were grown for 6 weeks prior to transplanting and inoculation, and left to grow for another 6 weeks after transplanting and inoculation. The TL isoline with both *I-3* and *Mi* performed better compared to the isolines without *I-3* and *Mi* in terms of plant height and weight, and resulted in lower gall ratings and nematode egg counts. When the plants were not inoculated with nematodes and Fol, there was no significant impact of *I-3* or *Mi* genes (in combination or separate) on the above mentioned variables.

DIGITAL MEDIA EDUCATION FOR EXTENSION CLIENTELE. **Robinson, Julie C.[1], T.L. Kirkpatrick[2].** [1] 2301 S University Ave, Little Rock AR 72204, [2]362 Highway 174 North, Hope, AR 71801.

In the constantly changing area of digital communication, we need to utilize the technology and resources available in order to reach internal and external audiences. In the current climate of Extension financial reality (i.e., reduced travel budgets, time, personnel and a growing clientele), it is increasingly important for Extension educators to utilize tools that extend the reach of Extension in the most efficient way possible. One effective teaching tool for the University of Arkansas Cooperative Extension Service is online learning, as an alternate method for educating Extension clientele. In 2014 Arkansas Extension developed an online interactive soil sampling and nematology course. The online course was launched to educate participants about how and when to survey soybean fields for nematodes. The Arkansas Soybean Promotion Board, in partnership with the Arkansas Nematode Diagnostic Laboratory, offered a no cost opportunity for County Agents, consultants, and growers to survey Arkansas soybean fields during the summer and fall for nematodes. In order to qualify for the free nematode assays, interested participants were required to enroll in an online course, complete the course lesson, pass the course quiz, and complete the course evaluation. The concept was to inform participants how to properly collect samples as a way of improving the accuracy and reliability of the nematode assay results. The self-paced online interactive course was developed, and hosted on a Moodle platform accessible via the Internet. Interactive narrated lessons, videos, and print materials were developed to be used in the course. In the first two years 88 participants enrolled in the course, and to-date 108 participants have enrolled in the course. Forty-four percent of participants indicated that participation in this course was their first time enrolling in an online course, and 92% of participants reported that they plan to use web-based learning tools in the future. Using online learning as the format was rated favorably, with a majority of the participants reporting the course format was just as effective as a traditional face-to-face course. When asked for general comments, the students reported that they would like to see an online learning format used more often and that participating in the course should save time and money on samples that are not collected and stored properly. To-date participants continue to enroll in the course, despite the incentive program no longer being offered, this could be an indication of preferred online learning. Due to the positive feedback from the course evaluations, the online course will continue to be made available indefinitely. A more advanced online nematology course is currently being developed based on this introductory course. We will share course development methods, enrollment success, and evaluation results. Evaluation instruments and processes developed to review digital media education efforts for both clientele and personnel are tools for moving forward with successful online courses.

ECOLOGICAL DETERMINANTS OF ENDOPARASITE PREVALENCE IN INDIAN PEAFOWL *PAVO CRISTATUS*. **Rubi, Josuva[1], Giza Rachel George[1], Shanthala Kumar[1], P. Sundararaj[1], Honnavalli N. Kumara[2], S.L. Hafez[3], and S. Nivitha[4].** [1]Unit of Nematology, Department of Zoology, Bharathiar University, Coimbatore – 641046, Tamil Nadu, India,[2]Salim Ali Centre for Ornithology and Natural History, Anaikatty, Coimbatore-641108, Tamil Nadu, India, [3]U of I Parma REC, 29603 U of I Lane, Parma, ID 83660, USA, [4]Kumaraguru College of Technology, Coimbatore, India.

The Indian peafowls are one among the heavy-bodied bird species facing severe threats due to the unavoidable anthropogenic activities. As they are an opportunistic feeder, omnivorous and have a wide range of foraging habitats, a chance of acquiring an endoparasite infection is apparent. Parasites have great pressure on the host due to their abundance and diversity, which leads to change in the behavior of the host by altering parasite load to complete the life cycle. This, in turn, leads to the death of the susceptible host and a decline in host population. Hence spatial-temporal dynamics of parasite population has been assessed by determining the species richness and species abundance with the variation in environmental conditions such as temperature and rainfall. A total of 18 species of parasites were recorded, that includes 13 Nematodes, three protozoans and two cestodes in 372 fresh fecal samples collected from 4 flocks of three study locations *viz*. Tamil Nadu Agriculture University (TNAU) Bharathiar University (BU), Institute of Forest Genetics and Tree Breeding (IFGTB) in Coimbatore, Tamil Nadu, India. The endoparasite infection was not affected by either temperature or rainfall. However, the abundance of endoparasite was significantly higher in monsoon than in post-monsoon and summer seasons. The abundance of oocysts of Coccidia spp. was widespread in all the seasons but significantly higher in monsoon than in post-monsoon and summer. The *Hymenolepis diminuta* populations was higher in adult peafowl than in juvenile birds. In contrast to this, infection of Coccidia spp. was greater in juveniles than in adults. The study provides first ever baseline data on endoparasite prevalence in peafowl of Coimbatore region, which is a hotspot for the peafowl. The study also reveals the variation in endoparasite infection across the seasons, which indicates monsoon is the more sensitive period for the peafowl. The information generated will be of useful for the management of the species if they are infected in the future.

THE STATUS OF PLANT-PARASITIC NEMATODES ASSOCIATED WITH POTATOES (*SOLANUM TUBEROSUM* L.) IN COSTA RICA: DISTRIBUTION, SPECIES IDENTIFICATION AND INTRASPECIFIC VARIABILITY OF *GLOBODERA* SPP. **Sandoval-Ruiz, Rebeca, L. Flores-Chaves, D. A. Humphreys-Pereira.** Laboratory of Nematology-CIPROC, University of Costa Rica, San Pedro, Costa Rica, 2060.

Plant-parasitic nematodes are one of the main phytosanitary problems affecting tuber quality and yields on potatoes in Costa Rica. However, the incidence and diversity of nematodes in potatoes has not been investigated in the last 35 years in the country. The objectives of this research were: (a) identify the plant-parasitic nematodes associated with potatoes in the main potato-growing areas of Costa Rica, (b) distinguish the species of the main nematode genera using molecular methods and determine their distribution in the country, and (c) determine the intraspecific variability of *Globodera* sp. Root and rhizophere soil samples (n = 25/field) were collected from fields in Cartago and Zarcero. Among the most frequently recovered nematodes on potato roots were *Pratylenchus* (62%), *Meloidogyne* (32%) and *Globodera* (26%). The relative frequency of *Globodera* cysts on soil was 70%. *Meloidogyne* was found at a range from 1609 to 2399 meters above sea level (m.a.s.l). *Pratylenchus* between 1840 and 2918 m.a.s.l. and *Globodera* between 1956 and 2886 m.a.s.l. *Globodera* cysts were detected between 1770 and 2918 m.a.s.l. *Meloidogyne* species were identified as *M. incognita* and *M. hapla* using PCR-RFLP. *M. incognita* was found at a range between 2083 and 1609 m.a.s.l., while *M. hapla* between 2186 and 1813 m.a.s.l. *Globodera* spp. were identified by sequencing the *ITS* nuclear marker and the mitochondrial marker cytochrome b (*cob*) from 12 populations. All *Globodera* populations were identified as *G. pallida* and the alignment of the sequences from both markers resulted in a unique haplotype. A BLAST search of the *ITS* sequence showed 99.7% - 100% identity to isolate P5A from South America, whereas the analysis of the *cob* marker showed a sequence similarity of 100% with an isolate from Otuzco, Perú, and 99.9% identity with isolates from Huancavelica, Huancayo, Jauja and Cajabamba. Phylogenetic relationships based on the *cob* gene placed the *G. pallida* haplotype from Costa Rica within a large clade composed of sequences from northern Perú, with high support (PP=100). The presence of only one haplotype for each marker, *ITS* and *cob*, the high sequence similarity and the phylogenetic relationships might indicate that the Costa Rican population of *G. pallida* has a unique origin, possibly from the northern regions of Perú.

PERFORMANCE OF THE BIONEMATICIDE MAJESTENE™ AGAINST PARASITIC NEMATODES IN TOMATO AND STRAWBERRIES IN FLORIDA. **Santos, Bielinski M.** Marrone Bio Innovations, 1540 Drew Ave., Davis, CA 95618, USA; email: bsantos@marronebio.com.

The bionematicide Majestene is a liquid formulation resulting from heat-killed cells of the new bacterium *Burkholderia rinojensis* strain A396, which contains several metabolites that prevent nematode molting and egg mass formation. A summary of six field studies conducted by universities and independent contractors is presented. Two studies were conducted in commercial strawberry fields in the 2015-16 season. The first study compared the efficacy of Majestene on parasitic nematodes at two rates and application frequencies (1 and 2 gal/acre applied once or twice through drip irrigation), as well as a commercial standard treatment (Inline; 1,3-dichloropropene + chloropicrin at 35 gal/acre), and a non-treated control. The

second strawberry study had similar treatments with the exception of Nimitz (fluensulfone at 0.4 mL/m row) as the commercial standard. The results indicated that application of Majestene at 1 or 2 gal/acre at 0 and 4 weeks after transplanting was comparable to both commercial standards on *Meloidogyne* spp. soil at 4 weeks after the last treatment. In tomato, several studies were conducted in 2015 and 2016 to compare control of *Meloidogyne* spp. with one or two drip-injections of Majestene at 9.5 and 19 L/ha, individually against metam potassium (=K-Pam or Sectagon) at 568 L/ha, *Paecilomyces lilacinus* strain 251 (=MeloCon) at 2.25 kg/ha, oxamyl (=Vydate L) at 4.7 L/ha. Data showed equal or superior control of juveniles and adults of southern root-knot nematode in all studies. These results indicate that Majestene is a new valuable tool to control troublesome plant parasitic nematodes, while reducing the risk for personnel exposure and pest resistance.

SOD-BASED ROTATION FOR *ROTYLENCHULUS RENIFORMIS* MANAGEMENT AND EFFECTS ON NEMATODE COMMUNITY STRUCTURE IN PEANUT-COTTON SYSTEMS. **Schumacher, Lesley[1], Z.J. Grabau[1], H.L. Liao[2], D.L. Wright[2], I.M. Small[2].** [1]Department of Entomology and Nematology, University of Florida, Gainesville, FL, 32611, [2] North Florida Research and Education Center, University of Florida, Quincy, FL 32351.

Cotton (*Gossypium hirsutum*) and peanut (*Arachis hypogaea*) are both major crops in the southeastern United States. Increased emphasis on sustainable agricultural practices leads growers to try to maximize their yields while maintaining soil health through practices such as sod-based rotation. In sod-based rotation, two years of pasture bahiagrass (*Paspalum notatum*) are followed by a year each of peanut and cotton. Sod-based rotation improves cotton yield, soil fertility, and water infiltration versus a conventional crop rotation of one year of peanut followed by two years of cotton. Reniform nematode (*Rotylenchulus reniformis*, RN) is an economically important pest of cotton while peanut and bahiagrass are non-hosts, so sod-based rotation may be an effective tool for managing this nematode. RN is known to infest deep in the soil profile but not much is known about free-living nematodes at deeper depths. Therefore, nematode population dynamics were investigated at different soil depths in sod-based and conventional rotations with or without irrigation at a long-term research site at the North Florida Research and Education Center. Soil samples were collected to 120 cm before planting in 2017 using a hydraulic probe and the nematode community was analyzed in 30 cm-sections. No irrigation effects on nematode abundances or indices were observed in the pre-plant samples in 2017 ($P>0.05$). RN abundance was greater in the conventional rotation than the sod-based rotation. Bacterivore abundances were greater following conventional peanut, sod-based peanut and sod-based cotton than conventional cotton or second-year bahiagrass. Channel Index was greater following conventional cotton than sod-based cotton and conventional peanut. RN decreased step-wise as soil depth increased, ranging from 5,139 RN/100 $cm^3$ soil at the 0-30 cm depth to 362 RN/100 $cm^3$ soil at the 90-120 cm depth in the soil profile. Bacterivore abundance also decreased as soil depth increased, ranging from 401 bacterivores/100 $cm^3$ soil at the 0-30 cm depth to 6 bacterivores/100 $cm^3$ soil at the 90-120 cm depth in the soil profile. Channel Index was greater at the 60-90 cm depth than the 0-30 cm depth in the soil profile. There were significant crop by depth interactions for fungivores and ring nematodes (*Mesocriconema xenoplax*), and there were biologically meaningful crop effects only at the 0-30 cm depth. At the 0-30 cm depth, fungivore abundance was significantly greater in second-year bahiagrass than any other crop phase. At the 0-30 cm depth, ring nematode abundance was greater in the peanut phase of sod-based rotation than all other crop phases except conventional peanut. Based on this research, nematodes are present at least 120 cm deep in the soil profile in these cropping systems, but are more abundant closer to the surface in spring before planting. Additionally, sod-based rotation is helpful for RN management and influences nematode community structure.

COMPARATIVE GENOMICS OF ENTOMOPATHOGENIC NEMATODES. **Schwartz, Hillel[1], L. Serra[2], M. Macchietto[3], A. Mortazavi[2], and P. Sternberg[1].** [1]Division of Biology and Biological Engineering, California Institute of Technology, Pasadena, CA 91125, USA, [2]Department of Developmental and Cell Biology, University of California, Irvine, CA 92697, USA, [3]Institute for Health Informatics, University of Minnesota, Minneapolis, MN 55455, USA.

Entomopathogenic nematodes (EPNs) disperse as infective juveniles to hunt their insect prey. Upon finding a host, they invade its body and release symbiotic pathogenic bacteria that rapidly kill the insect and convert it into a food source, while preventing colonization of the carcass by other microorganisms and predation by other scavengers. This lifestyle has arisen independently at least three times, in two different clades: the *Steinernema* and *Heterorhabditis* nematodes, and a subset of *Oscheius* nematodes. To better understand how these nematodes have converged on this shared strategy, we have sequenced the genomes and transcriptomes of five species of *Heterorhabditis*, to compare with our previous examination of the genomes and transcriptomes of five species of *Steinernema*. We are seeking genomic traits common to these nematodes and in particular to the infective juveniles of these nematodes. Such traits might represent differences with free-living or with parasitic nematodes, some of them more closely related to these EPNs but not sharing their symbioses or lifestyle. Comparisons of these species may reveal convergent evolution to use mechanisms that regulate responses to bacterial interactions and variations that correlate with differences in lifestyle or bacterial compatibility. In addition to genomic studies of the different EPN species, we hope to develop *H. bacteriophora* as a laboratory organism. *H. bacteriophora* grows well on plates, is reportedly susceptible to RNAi and transgenesis, and can develop as selfing hermaphrodites, and so should be a powerful system for molecular genetic study of symbiosis. When cultured at low density these nematodes develop almost

exclusively as females. We have screened for and isolated a constitutively hermaphroditic mutant for use in molecular genetic studies of symbiosis and of sex determination.

COMPARITIVE GENOMICS OF ENDODERM CELL FATE SPECIFICATION IN *STEINERNEMA CARPOCAPSAE*. **Serra, Lorrayne[1], M. Macchietto[2], B. Rodriguez[1], C. McGill[1], A. Mortazavi[1].** [1]Department of Developmental and Cell Biology, University of California Irvine, Irvine CA**.** [2]Institute of Health and Bioinformatics, University of Minneapolis, Minneapolis, MN.

We are interested in using entomopathogenic nematodes (EPNs) from the family *Steinernematidae*, which are nematodes that parasitize and efficiently kill insects and are used as satellite model organism, to study the conservation of endoderm development in nematodes. The *Steinernema carpocapsae* genome lacks the GATA transcription factors END-1 and END-3, which control endoderm development in the E-cell of the 8-cell stage in *C. elegans* while their downstream target genes are conserved and expressed abundantly during endoderm development. Therefore, there must be an alternative set of early-expressed Transcription Factors (TFs) that determine endoderm cell fate in *S. carpocapsae*. We are isolating single-cells from *S. carpocapsae* and have sequenced individual single-cells from early stages of *S. carpocapsae* to identify early zygotic TFs that could be cell lineage specific. The embryonic localization of these TFs will be verified using single molecule fluorescent *in situ* hybridization(smFISH). We will compare our results in S. carpocapsae to the matching single-cell data from *C. elegans* E-cells to perform the first comparison of gene expression at the single-cell level among homologous cells across distant nematode species with a focus on regulatory genes controlling early endoderm development.

QUILLAJA SAPONIN INCREASES LONGEVITY AND REDUCES LIPID ACCUMULATION IN *CAENORHABDITIS ELEGANS*. **Shanmugam G.[1], M. Loganayagi[1], A. Mohankumar[1], P. Sundararaj[1], S.L. Hafez[2] and S. Nivitha[3].** [1]Unit of Nematology, Department of Zoology, Bharathiar University, Coimbatore, [2]U of I Parma REC, 29603 U of I Lane, Parma, ID 83660, USA, [3]Kumaraguru College of Technology, Coimbatore, India.

Experiments were carried out to investigate the antiaging, hypolipidemic and antioxidant gene activity of Quillaja saponin (QS) on the nematode model *Caenorhabditis elegans*. QS from soap bark tree (*Quillaja saponaria* Mol.) is a vaccine adjuvant, anti-inflammatory agent, analgesic agent and also used in food beverages. Eggs of *C. elegans* were collected from one day old adult worms treated with sodium hypochlorite solution (5%NaClO and 5N NaOH). L1 larvae hatched out from the eggs were synchronized overnight at 20°C by using M9 buffer (3 g KH2PO4, 6 g Na2HPO4, 5 g NaCl, 1 ml 1 M MgSO4, H2O to 1 litre) and transferred to NGM plates (1.7% Agar, 2.5g Cascein Peptone, 3g NaCl) which contain *E.coli* OP50 and cultured for 48 hours to obtain L4 stage. To find out the toxic dose, L4 worms were transferred into 48 well plates and kept for 24 hours at 20°C in S-Basal solution (5.85 g NaCl, 1 g K2 HPO4, 6 g KH2PO4) which contain either 10-1000μg/ml QS (Dissolved with 50%Ethanol) or ethanol that served as solvent control. Since the concentration of QS above 500μg/ml was toxic to *C. elegans*, further studies were carried with three lower concentrations *viz*. 100, 300 and 500μg/ml. Antioxidant activity of QS was carried out under in vitro conditions by using DPPH free radical scavenging assay and found 76.4μg/ml QS inhibit 50% of free radicals (IC50). Lifespan studies of N2 worms indicated that there was an increase in the lifespan of 8.16, 16.6 and 22.1% over control under 100, 300 and 500μg/ml respectively. For the stress resistance studies, the worms were pretreated with respective concentrations for up to 2 days and then exposed to the pro-oxidant juglone (250μM) for 5 hrs. Mortality of worms in each concentration was measured by counting the dead worms. QS significantly increased the resistance to oxidative stress and thermal stress at 35°C. It was further confirmed that expression level of antioxidant genes *sod-3(CF1553)* and *gst-4(CL2070)* were increased significantly ($p>0.05$). Expression of heat shock responsive gene *hsp-16.2* was reduced significantly ($P>0.01$) as visualized by GFP reporter by fluorescence microscope under blue wavelength. To analyse the hypolipidemic activity, the worms were first exposed to 50mM glucose from L1 to adult day 2 with or without 500μg/ml QS. Lipid staining by Oil O Red confirmed that QS reduced accumulation of lipids significantly ($P>0.001$). All these studies confirmed that QS had ROS scavenging activity, mild stress inducing ability and hypolipidemic activity in *C. elegans*.

ADVANCING APPLICATION OF ENTOMOPATHOGENIC NEMATODES IN ORCHARD CROPS. **Shapiro-Ilan, David**. USDA-ARS, Southeastern Fruit and Tree Nut Research Laboratory, Byron, GA 31008.

Through our research, we have made several advancements in developing entomopathogenic nematodes for control of insect pests in orchard crops. Our research focuses primarily on pecan and peach cropping systems. However, the innovative methods that we have developed will be applicable to other systems as well. Key pests that were studied for development of entomopathogenic nematode control tactics included pecan weevil (*Curculio caryae*) in pecan, and plum curculio (*Conotrachelus nenuphar*), lesser peachtree borer (*Synanthedon pictipes*) and peachtree borer (*Synanthedon exitiosa*) in peach. Our results indicate that high levels of control can be achieved against each of these pests using biological control methods. For pecan weevil, an integrated program was tested using entomopathogenic nematodes (*Steinernema carpocapsae*), entomopathogenic fungi (*Beauveria bassiana*), and the bacterial-based product Grandevo® (*Chromobacterium subtsugae*); also, a cumulative control approach using S. *carpocapsae* (over the life-cycle of the insect) was measured. For peachtree

borers, we applied the nematode, *S. carpocapsae* in the late summer and fall as a preventative application for *S. exitiosa*, and also tested *S. carpocapsae* as a curative treatment for *S. exitiosa* and *S. pictipes* by applying the nematodes in the spring to borer-infested trees. Additionally, a novel fire-gel formulation was used to protect nematodes from adverse environmental conditions. For plum curculio, a sentinel tree (trap-tree) approach was tested that reduces substantially the area that must be treated. Results from the pecan weevil experiments indicated that an integrated microbial control approach (using entomopathogenic nematodes, fungi and Grandevo®) caused significantly lower nut damage compared with the non-treated control. Cumulative control of *S. carpocapsae* resulted in less than 1% survival of the pecan weevil. In the peachtree borer experiments, the nematode, *S. carpocapsae* reduced *S. exitiosa* infestations in both preventative and curative applications. The level of control observed was equal to or greater than standard chemical insecticides (chlorpyrifos). For *S. pictipes*, damage was also reduced in a manner equal to the use of standard chemicals. Furthermore, the nematodes were effective when applied using various standard spray equipment (boom, trunk sprayer, or handgun). In the plum curculio experiments, high levels of control were observed using the nematode *S. riobrave*. The trap-tree method has potential for a successful biocontrol approach in managing plum curculio. In conclusion, based on novel application approaches, entomopathogenic nematodes were highly effective in controlling pecan weevil, peachtree borer, lesser peachtree borer and plum curculio.

PERSISTENT NATIVE EPN STRAINS: AN INOCULATIVE APPROACH FOR MULTI-YEAR SOIL INSECT CONTROL. **Shields, Elson J. and A.M. Testa.** Department of Entomology, Cornell University, Ithaca, NY. 14853.

Entopathogenic nematodes (EPNs) have been isolated from every inhabited continent, in virtually every soil habitat where a concentrated effort has been made to find them, thriving under local conditions and evolving to exploit local fauna. Naturally occurring EPNs survive unfavorable conditions through the use of phased infectivity to persist across shortages of available hosts and unfavorable temperatures. When the natural habitat is disrupted with agriculture, the native EPNs may not be able to suppress the new array of insects attacking the introduced agricultural crops through a mismatch in soil overlap or susceptibility of the host. To test the concept of utilizing these naturally occurring EPNs in agricultural pest management in a more classical biological control approach, NY strains of *Steinernema carpocapsae* (NY 001),and *S. feltiae* (NY04) were isolated from agricultural fields in Northern NY and cultured to preserved the characteristics which allow them to persist in the NY climatic conditions. *S. carpocapsae* and *S. feltiae* in a species mix, were applied to 85 alfalfa fields in Northern NY at an inoculation rate of 250 million IJs per species per ha in 475 L water per ha. EPN populations were followed in each field across rotations consisting of multi-year alfalfa and multi-year corn rotations. In all fields and all crops, EPN populations persisted and responded to insect invasion. Between invasions, EPN populations persisted in alfalfa between 27-43% of the soil cores bioassaying positive for EPN. In corn rotated from alfalfa, EPN populations persisted between 28-55% of the soil cores positive for EPNs. Upon insect invasion, the frequency of EPN increased in many instances to 100% of the soil cores positive for EPNs. In corn fields, the increase of EPN populations correlated with corn rootworm. *Diabrotica virgifera virgifera,* invasion and larval hatch. In some fields, both corn and alfalfa, the dramatic increase of EPNs in response to insect invasion occurred 5-9 years after the single inoculation. In a field experiment focused on EPNs and corn rootworm, first year corn was inoculated with EPNs in June 2014 and became established. In 2016, the established EPNs provided protected the corn roots from corn rootworm larval feeding equal to or exceeding the three common Bt-Rootworm corn varieties. Our data clearly suggests that the native EPN isolates in the proper species mix, can be inoculated into agricultural soils planted to alfalfa/corn rotation a single time and they will persist at a moderate level in the field for multiple growing seasons. When the field is invaded with susceptible insects, the resident population of EPNs respond and prevent economic crop damage.

EVALUATION OF POTENTIAL TRAP CROPS FOR MANAGEMENT OF *HETERODERA GLYCINES* IN MICHIGAN SOYBEAN PRODUCTION SYSTEMS**.** **Shoemaker, Jeff, and G. Bird.** Department of Entomology, Michigan State University, East Lansing, Michigan.

Soybean cyst nematode (SCN), *Heterodera glycines,* is a key pest of soybeans in Michigan. While management options such as resistant varieties, seed treatments, and crop rotations are available, there is a distinct need for additional control practices, especially in regards to the prevention of development of highly aggressive populations. Cover crops are often used in managing soil health issues and specific cultivars are used in Michigan as trap crops for management of sugar beet cyst nematode (BCN), *Heterodera schachtii*. The objective of this research is to evaluate selected cover crop cultivars and blends for potential as trap crops for SCN . Experiments were conducted under greenhouse conditions and in three field locations in Michigan (Ingham, Monroe, and Cass counties). Eleven cultivars from two genera (*Raphanus* spp. and *Sinapis* spp. of the Brassicaeae (Cruciferae) were evaluated in the greenhouse experiments, including one multi-cultivar blend. Six were selected for the field experiments. All trials included a SCN susceptible and SCN resistant soybean cultivar. A fallow treatment was included in the field trials. After 45 days under greenhouse conditions, all eleven cultivars in the greenhouse experiments had significantly lower egg and cyst population densities than the susceptible control. This was not always true in regards to the number of eggs per cyst, which was not significantly different from the susceptible control. The field research results varied among locations. In Monroe and Cass counties, all cover crop cultivars resulted in fewer eggs, cysts, and eggs/cyst, compared to the susceptible control. In Ingham country, however, there were significant differences between the cover crop cultivars

and the susceptible control for all three SCN life stage indicator categories. While progress has been made in our research in regards to a trap crop for SCN, more experimentation needs to be done to determine if potential cultivars are true trap crops or if they are only poor or non-hosts.

MOLECULAR AND MORPHOLOGICAL CHARACTERIZATION OF PLANT-PARASITIC NEMATODES FROM GREECE. **Skantar, Andrea M.[1], Z.A. Handoo[1], M. Kormpi[2], and E.A. Tzortzakakis[3].** [1]Mycology and Nematology Genetic Diversity and Biology Laboratory, USDA-ARS, 10300 Baltimore Ave., Bldg 010A BARC West Rm. 113, Beltsville, MD 20705; [2]Benaki Phytopathological Institute, Athens, Greece; [3]Subtropical Crops and Viticulture, N.AG.RE.F., Hellenic Agricultural Organization-DEMETER, Heraklion, Crete, Greece.

The occurrence of *Heterodera* spp. was investigated in soil samples collected from fields of potato, beet, and other crops in Greece. Molecular and morphological studies were conducted to identify several species, including *H. carotae, H. goettingiana, H. schachtii, H. trifolii, H. latipons, H. filipjevi,* and *H. avena*e. Species identifications were based upon molecular phylogenetic trees constructed using sequences from the internal transcribed spacer (ITS) and 28S rDNA regions. In many instances, morphological analyses were in clear agreement with molecular data, while a few species were resolved by molecular analysis when morphometrics were overlapping or unresolved. An expanded phylogenetic analysis of Hsp90 sequences was able to further resolve some species boundaries and should aid future molecular diagnostics of these cyst nematodes. *Heterodera trifolii, H. goettingiana*, and *H. filipjevi* represent new country records for Greece.

SEED-BORNE NEMATODES OF PEANUT IN SOUTHERN AFRICA. **Steenkamp, Sonia[1], H. Fourie[2], and A. Swart[3].** [1]ARC-Grain Crops Institute, Private Bag X1251, Potchefstroom, 2520, South Africa, [2]North-West University, Unit for Environmental Sciences and Management, Private Bag X6001, Potchefstroom, 2520, South Africa, [3]Nematology Unit, Biosystematics, ARC-Plant Protection Research Institute, Private Bag X134, Queenswood 0121, South Africa.

Peanut is primarily consumed as a vegetable by small-scale farmers and produced as a cash crop by commercial farmers in South Africa. It is cultivated under both rain-fed conditions and irrigation in the summer rainfall regions. Three seed-borne nematode species have been identified on peanut produced in South Africa, of which *Ditylenchus africanus* is currently believed to be the most economically important. *Ditylenchus africanus* is present throughout the peanut production area and its effect on peanut is mainly qualitative, causing downgrading of peanut consignments and consequently, financial losses for the peanut industry. Symptoms on *D. africanus* infected peanuts include pods with darkened veins and dark areas, while the seeds show dark, discolored testae, sometimes with visible darkened veins. Premature germination of seeds sometimes occur even before the producer is able to harvest. The second species, *Aphelenchoides arachidis*, was identified from peanut hulls and kernels from the Vaalharts Irrigation Scheme (Northern Cape Province) and Vryburg (North-West Province) areas during 2002. The spread of the latter nematode is localized and its presence in localities other than Vaalharts and Vryburg is not known at present. Damage symptom of *A. arachidis* is similar to that of *D. africanus*. Large numbers of a third nematode species belonging to the Aphelenchoididae, were found on peanut kernels and hulls from the Vaalharts Irrigation Scheme area during May 2016. Numbers of this nematode ranged from 2 – 1 531 nematodes per 5 g seed and 10 – 22750 nematodes per 5g hulls. Symptoms include blackish discoloration of the hulls and discolored and shrunken seed. Future research on this nematode will include a survey to determine if the species is present in localities other than Vaalharts, determining its reproduction potential and its aggressiveness to peanut and other crops used in rotation with peanuts.

PROFILING SOIL FREE LIVING NEMATODE COMMUNITY IN AN EXTREME DESERT ECOSYSTEM – NAMIBIA. **Steinberger, Yosef[1], G.Maggs-Kölling[2], E. Marais[3], C. Sherman[1], T. Doniger[1].** [1]The Mina & Everard Goodman Faculty of Life Sciences Bar-Ilan University, Ramat-Gan, 5290002, Israel, [2]Gobabeb Research and Training Centre, Namibia, [3]Department of Entomology, National Museum of Namibia, Windhoek, Namibia.

Functional structure and diversity of soil free living nematode community in a desert xeric environment may depend on plant gender and its location along a gradient from sea shore to inner desert. The objective of the present study was to compare soil free living nematode community from the upper 0-10 cm soil horizon beneath *Acanthosis horridus* male and female plants. The soil samples collected were analyzed for soil moisture (SM), organic matter (OM), pH and soil free living nematode community. Nematode community included, total number and, molecular analysis using the 18S rRNA gene for species determination, that was found to be a useful tool for evaluation of ecological conditions under the plant associations. The soil free living nematode community were found to be affected by both by sampling location and plant gender along transect from the west site to inner desert of Namibia. Moreover, the used of the molecular tools had found to be greatly useful in defining the waste richness of animal parasite nematodes present as result of animal food and water foraging. In summary it was consistent the importance of *A. horridus* in determining microhabitat contribution of abiotic variables which had determined soil free living nematode community composition, abundance and trophic composition.

GENETIC IMPROVEMENT OF OXIDATIVE STRESS TOLERANCE AND LONGEVITY OF THE ENTOMOPATHOGENIC NEMATODE *HETERORHABDITIS BACTERIOPHORA* DAUER JUVENILES. **Sumaya, Nanette Hope[1, 2], B. Vandenbossche[2], M. Barg[2], V. Doerfler[2], O. Strauch[2], C. Molina[2] and R.-U. Ehlers[1, 2].** [1]Faculty of Agricultural and Nutritional Sciences, Christian-Albrechts-University Kiel, Hermann-Rodewald-Str. 4, 24118 Kiel, Germany, [2]e-nema, GmbH, Klausdorfer Str. 28-36, 24223 Schwentinental, Germany.

The commercial use of the entomopathogenic nematode (EPN) *Heterorhabditis bacteriophora* as a biocontrol agent against noxious insects in the field of agriculture is limited due to its relatively short shelf-life. Longevity of dauer juveniles (DJs) during storage and in-transit to end users is restrictive. Both DJ-longevity and persistence in the field are influenced by temperature, desiccation, and other environmental stress factors. Recent studies have demonstrated the potential of genetic selection to improve heat- and desiccation tolerance in *H. bacteriophora*. In this study, a direct link between oxidative stress-, desiccation-tolerance and DJ-longevity was observed. Oxidative stress tolerance of *H. bacteriophora* DJs in a wide collection of strains and inbred lines from different origins were characterized at two different temperature regimes and compared it to the mean time survival ($MTS_{50}$) without oxidative stress induction. A significant correlation between DJ-survival under oxidative stress and the DJ-longevity during storage in tap water was recorded. Moreover, it was observed that oxidative stress overrides the prolonged lifespan of *H. bacteriophora* DJs held under low temperature. The heritability ($h^2>0.9$) of the oxidative stress tolerance in DJs suggests a high probability of success for improvement upon selective breeding. Thus, the $MTS_{50}$ of DJ populations under oxidative stress can be used as a predictor of DJ-longevity in *H. bacteriophora*. As proof of principle, a subset of *H. bacteriophora* inbred lines with contrasting oxidative stress tolerance was characterized for DJ-survival after application (persistence). The DJs with high oxidative stress tolerance persisted longer and were further able to infect *Tenebrio molitor* larvae assessed in petri dishes using sand assays. Parallel to screening natural materials, *H. bacteriophora* Ethyl methanesulfonate (EMS)-mutants were generated and subjected to oxidative stress as a selection parameter for enhanced DJ-longevity. The $MTS_{50}$ of selected EMS-mutants was increased by at least five days compared to the donor line. In addition, part of the progeny obtained through genetic crosses of lines with high DJ-longevity outperformed the parental lines, signifying the additive effects. To gain knowledge about the underlying genes involved in stress tolerance in *H. bacteriophora*, the oxidative stress-responsive transcriptome of low- and high-surviving lines was analysed and candidate genes were screened for polymorphisms. Approximately 400 SNPs have been detected between the two inbred line groups. PCR-based genotyping markers were derived from relevant transcripts and were tested in natural *H. bacteriophora* materials. Significant correlation between genotype and phenotype was determined for a subset of transcripts. The results of this study open a new area of research for the understanding of the DJ-longevity in EPN.

DETECTION AND IDENTIFICATION OF THE FIG CYST NEMATODE, *HETERODERA FICI* KIRJANOVA, ON FIG TREE, *FICUS CARICA*, IN ONTARIO, CANADA. **Sun, Fengcheng[1], N. Henry[1], Q. Yu,[2].** [1] Canadian Food Inspection Agency, 3851 Fallowfield Road, Ottawa, ON, K2H 8P9, Canada, [2] Ottawa Research and Development Center, Agriculture and Agri-Food Canada, Ottawa, ON K1A 0C6, Canada.

The fig cyst nematode, *Heterodera fici* Kirjanova, is a pest of fig plants. Heavy infestation can cause retarded growth and yellowing of leaves. In the spring of 2016, during an inspection, a sample from rhizosphere of a potted fig (*F. carica*) seedling from a nursery in Niagara-on-the-Lake, Ontario, Canada, was submitted to the Nematology Laboratory, Canadian Food Inspection Agency. The fig trees in the nursery had been grown in the fields during the growing seasons and potted and moved indoors during the winters for the past three years. Nematodes were extracted using decanting, sieving and misting. Lemon-shaped cysts and second-stage juveniles of *Heterodera* sp. were recovered. The morphological and molecular analyses of the cysts, vulval cones, and second-stage juveniles from both the roots and the crushed cysts identified the species as *Heterodera fici* Kirjanova. For molecular analysis, DNA was extracted from individual juveniles (n=4) from different cysts. A 1,151-bp fragment of ribosomal DNA containing ITS1-5.8S-ITS2 region was amplified and sequenced. The sequence was compared with published sequences by means of a NCBI BLAST search in the database. The comparison revealed 99.0 to 100% similarity to the published sequences of the same genomic region of *H. fici*. The fig cyst nematodes have been reported from Belarus, Belgium, Estonia, France, Germany, Greece, Hungary, Italy, The Netherlands, Norway, Poland, Portugal, Russia, Spain, Yugoslavia, China, Georgia, Iran, Turkey, Uzbekistan, Australia, New Zealand, USA (California, Florida, Louisiana, Maryland, Virginia), Brazil, Algeria, and South Africa. It is an exotic pest to Canada. This is the first evidence of the occurrence of *H. fici* in Canada.

EXAMINATION OF MAIZE ROOT EXUDATES ON SOYBEAN CYST NEMATODE. **Taylor, Christopher G., and R. Medina.** Department of Plant Pathology, The Ohio State University, Wooster, OH 44691.

Soybean cyst nematode (*Heterodera glycines;* SCN) is mainly managed through the use of resistant soybean cultivars and non-host crop rotations. Recently, resistance-breaking populations of SCN have made the use of SCN-resistant cultivars far less effective in controlling this nematode. Reliance on crop rotation has now become significantly more important for SCN

control. However, little is known about how widely used non-host crops including corn and wheat, can impact SCN populations. Previous reports suggest that SCN numbers can be reduced during rotations with maize but the mode of action in this interaction is unknown. This study aimed to identify maize lines from the Nested Associated Mapping series of maize breeding population that are most suitable for decreasing SCN populations through the production of allelopathic chemicals in root exudates. Collected root exudates were screened for their ability to affect SCN hatching. Of the 27 maize lines tested, we observed lines that repeatedly altered SCN hatching rates. This exudate-induced variety of hatching responses suggest that phenotypic differences occur between maize lines and that these genetic factors that regulate these differences might be identifiable through breeding and/or gene expression analysis. More immediate benefits of this research is to identify maize lines that could be used in rotation with soybean to help reduce SCN numbers in the field.

SPATIAL NEMATODE COMMUNITY DISTRIBUTIONS ASSOCIATED WITH HUMAN DECOMPOSITION IN A MASS GRAVE. **Taylor, Lois S., G. Phillips, S.W. Keenan, E.C. Bernard, J.M. DeBruyn.** Biosystems Engineering & Soil Science, University of Tennessee, Knoxville, TN 37996.

Human cadaver decomposition is a dynamic process that results in localized soil nutrient enrichment. During mass loss, decomposition products and microflora enter the soil where successional shifts in microbial communities have been documented as diverse taxa compete for temporally changing pools of resources. While these successional shifts have been primarily studied for bacteria (via 16S rRNA metagenomic profiles), it is anticipated that these shifts in resources have the potential to affect microfauna present in the local food web that use these bacteria as food sources. Nematode diversity is a sensitive indicator of soil enrichment and structure; therefore, nematode community responses in decomposition environments may prove useful for determining decomposition progress and ultimately estimation of post-mortem interval (PMI). The objective of this study was to evaluate soil nematode communities under and around buried human cadavers at the University of Tennessee Anthropology Research Facility. Three human cadavers were buried in a single grave at a depth of 30-70 cm and allowed to decompose naturally for four years. Soil samples were collected at 50-cm intervals for a total distance of 2m along transects radiating away from the grave at depths of 0-10 cm and 30-40 cm. During grave excavation, soils were collected from the top 10 cm of the grave, at the 30-40-cm cadaver level and beneath the grave at depths of 70 and 85 cm. Soil nematodes were extracted by sugar flotation-centrifugation and characterized to genus level. Soil nematode community richness in transects exhibited a marked reduction (84%) between surface soils and those at the 30-cm depth. Soils collected at 85 cm contained no detectable nematodes. Richness in soil samples within the grave area was highly variable at all depths; in two of four grave samples the richness at 30 cm exceeded that of the surface soils. Evenness between surface and 30 cm soils outside the grave varied by 12%, increasing with depth. Grave soil evenness followed a reverse trend, decreasing by 30% with depth. Shannon diversity at 0 and 30 cm depths was similar outside the grave; however, soils inside the grave varied considerably. All surface soil samples were categorized as disturbed according to Ferris faunal profile analysis. In contrast, populations from the 30-cm depth were characterized as maturing with balanced or low disturbance. All other depths had negligible structure and were classified as extremely disturbed or distressed enrichments. Overall, alpha and beta diversity and faunal profiles within the grave diverged markedly by depth compared to soils outside the grave. This divergence may reflect persistent effects of cadaver-introduced soil enrichment, including the localized presence of adipocere, as well as soil disturbances brought about by mixing of soil profiles during interment. This study constitutes a proof-of-principle of the hypothesis that nematode community structures are altered for extended time periods in decomposition environments.

SOIL AMENDMENTS WITH EXTRACTED JUICES AND OILS OF FIVE PLANT SPECIES OF CITRUS FRUITS FOR THE CONTROL OF *MELOIDOGYNE SPP* ON TOMATO UNDER FIELD CONDITIONS. **Tefu Grace, M.S. Daneel, W.P. Steyn, C.S. Arries and T.D. Selabela.** ARC-Institute for Tropical and Subtropical Crops, Private Bag X11208, Nelspruit 1200, South Africa.

Previously glasshouses experiments were conducted to evaluate the effect of soil amendments with extracted juices (grapefruit, lemon, sweet orange and naartjie) and oils (Lemon, lime and orange) for the control of *Meloidogyne incognita*. The organic amendment consisting of lemon juice gave the best reduction of nematodes but had no positive impact on yield. Orange juice persistently gave the best improvement in plant growth. Oils consistently performed weaker than the juice. Further studies have been carried out to confirm the potential of these organic amendments on the control of *Meloidogyne* spp. in the field. The field was naturally infested with a mixture of *M. incognita* and *M. javanica*. The trial was designed in completely randomised blocks. Similar juice extracts used in the glasshouse experiment were applied in the field @ 50ml/plant to determine the effect of the amendments on nematode control and yield. Considerable reduction of rootknot nematodes was achieved with lemon juice extract which compared well with standard nematicides. On the other hand orange juice extract gave the highest plant growth. Trials in the field are continuing to determine the long term effect of the amendments on nematode control and yield enhancement.

SEAM CELL PROLIFERATION IS ASSOCIATED WITH BODY SIZE DEVELOPMENT IN POST-INFECTION *HETRODERA GLYCINES*. **Thapa, Sita and N.E. Schroeder**. Department of Crop Sciences, University of Illinois, Urbana, IL, 61801.

The cyst nematodes (*Globodera* spp., *Hetrodera* spp.) are economically important pathogens. Infective J2s of cyst nematodes penetrate the host root, establish a feeding site, become sedentary and develop to J3, J4, and adult stages. Unlike most nematodes, cyst nematodes grow disproportionately greater in width than length. For example, the soybean cyst nematode *Heterodera glycines* develops from a newly hatched vermiform J2 into a lemon shaped adult female. Males initially increase in width until the final molt when they return to a vermiform shape. We are examining *H. glycines* post-infection development to better understand its unusual growth. It is reported that in *Caenorhabditis elegans* and other closely related nematode species the epidermis is the single most important organ in the development of body size. Body size evolution in some free-living nematodes is associated with alterations in seam cell lineage and ploidy level, and the number of epidermal nuclei. Seam cells are elliptical stem-cell like epidermal cells found along the lateral midline epidermis. In adult *C. elegans*, there are 16 seam cell nuclei on each side of the animal. We examined seam cell proliferation in *H. glycines* post infection for correlations with body growth. We observed seam cell development in *H. glycines* from the J2 to J3 stage and organized them according to age, based on the number of days post-inoculation (DPI) and gonad size. We identified ten seam cells in freshly hatched J2s and two DPI J2s. By four DPI we began observing seam cell division producing epidermal nuclei in *H. glycines*. Unlike *C. elegans* seam cells, which typically divide only once during each post-embryonic stage, *H. glycines* seam cells divide more than four times within the J2 stage alone. Seam cell daughter cells are oriented dorso-ventrally and subsequently migrate dorsally and ventrally away from the lateral midline. By the J2-J3 molt, 18 seam cells were observed in the lateral epidermis. We counted DAPI stained subdorsal and subventral epidermal nuclei from the pharynx to anterior of the tail before and after migration of seam cell-derived epidermal cells. On average, seven epidermal nuclei were observed prior to seam cell division. In total, 72 seam cell-derived epidermal nuclei were added before the J2-J3 molt. In *C. elegans*, only 14 seam cell-derived epidermal nuclei are added during the J2 stage. We found 113 epidermal nuclei in the J3 *H. glycines* males and 216 in J3 females. We observed a similar increase in the number of epidermal nuclei from the J3 to J4 female. We are currently utilizing immunohistochemistry and laser ablation of seam cells to better understand the *H. glycines* epidermis and its unusual growth pattern.

BIOLOGICAL ATTRIBUTES OF DUPONT™ SALIBRO™ A NOVEL SULFONAMIDE NEMATICIDE. **Thoden, T.C.[1], P. Link[1], F. Clappers[1], M. Rivera[2] and J.A. Wiles[3]**. [1]DuPont de Nemours (France) S.A.S., European Research Center; 68740 Nambsheim, France. tim.thoden@dupont.com, [2]DuPont Crop Protection, Stine-Haskell Research Center, 1090 Elkton Rd., Newark, DE 19714, USA, [3]DuPont (U.K.) Limited, 4th Floor, Kings Court, London Road, Stevenage, SG1 2NG, United Kingdom.

DUPONT™ SALIBRO™ is a novel sulfonamide nematicide containing the active ingredient fluazaindolizine (Vellozine™). In the past few years numerous laboratory studies have been performed to characterize its biological attributes and to optimize its fit with growers needs. This included a wide range of *in-vitro* studies on plant-parasitic nematode species to evaluate effects on nematode activity, mobility and plant infectivity. Abiotic factors such temperature and exposure time were also investigated. Species sensitivity differed strongly, and was highest for various species of root-knot nematodes, but also other important plant parasitic species such as root lesion, reniform, potato cyst or spiral nematodes were adversely affected. Nematodes exposed to fluazaindolizine showed strongly reduced activity and mobility coupled with a loss of infectivity within 24-72 hours of exposure to fluazaindolizine at 5-250 ppm. These effects were irreversible and only marginally affected by temperature. In contrast, no adverse effects were observed on numerous species of bacteriophagous or fungivorous nematodes at similar concentrations. This selectively against plant-parasitic species, together with the favorable mammalian and ecotoxicology safety profile of the molecule, make DuPont™ Salibro™ a useful future tool for nematode management.

SUPPRESSION OF CRICONEMATID-INDUCED INJURY TO GOLF COURSE GREENS IN NEW MEXICO. **Thomas, Stephen[1], J.M. Beacham[1], and T.O. Powers[2]**. [1]Department of Entomology, Plant Pathology and Weed Science, P.O. Box 30003 MSC 3BE, New Mexico State University, Las Cruces, NM 88003; [2]Department of Plant Pathology, 406 Plant Science, University of Nebraska-Lincoln, Lincoln, NE 68583.

Recent observations of turf decline in New Mexico support Criconematids as the principal plant-parasitic nematodes associated with such decline, which differs from observations in the southeastern USA where *Belonolaimus longicaudatus* and *Hoplolaimus* spp. are predominant turf pathogens and the western USA where several species of *Meloidogyne* and *Anguina pacificae* are often damaging. In summer 2011 the NMSU Nematode Diagnostic Laboratory received soil samples from numerous greens that were experiencing severe turf decline at the University of NM North Golf Course rendering these greens unsuitable for normal play. Samples yielded up to 9,520 Criconematids and 80 *Longidorus breviannulatus* per 100 $cm^3$ soil. Subsequent attempts to suppress nematode populations with *Bacillus firmus* (strain I-1582; Nortica®) in fall 2012 and spring 2013 reduced Criconematid numbers 21% and 73%, respectively, but the population remained at 2,140/100 $cm^3$ soil and condition of the turf remained unsuitable for normal golfing. Beginning in fall 2014 and continuing through spring

2015 greens were treated with 2% Abamectin (Avid®). Numbers of Criconematids were subsequently reduced to 140/100 $cm^3$ soil and *L. breviannulatus* to undetectable levels. By mid-summer 2015 turf on all treated greens had recovered to where no visual signs of the previous damage were evident and normal play could resume. During the same period when Criconematid populations were decreasing, numbers of *Pratylenchus* spp. increased nearly10-fold from those originally reported in 2011 to 390/100 $cm^3$ soil, despite continued Abamectin applications. Late summer applications of fluensulfone (NimitzProG®) in 2016 reduced *Pratylenchus* numbers by 50%. Similar incidences of turf decline in several additional golf courses in Albuquerque, NM, as well as Las Vegas, NV and Carlsbad, NM have been associated with Criconematid populations of 1,400 or more per 100 $cm^3$ soil. Unlike results from golf courses in AZ, CA, NV, UT, and WA no *Meloidogyne* species were detected from any courses in NM, but were recovered from the Las Vegas, NV sample. Criconematids should be considered as possible causal agents associated with turf decline in the Southwest.

EVALUATION OF VARIOUS COMMERCIAL AGRICULTURAL INPUTS FOR A COMPLETE MANAGEMENT SYSTEM OF *MELOIDOGYNE INCOGNITA* INFESTED FIELD CORN. **Till, Stephen, K.S. Lawrence,** Dept. of Entomology and Plant Pathology, Auburn University, AL, 36849.

*Meloidogyne incognita,* the southern root-knot nematode, causes significant yield losses in the major row crops across the southern United States. Corn is an important rotational crop in the southeast, and growing corn in a root-knot nematode infested field increases population density; thus, diminishing yield of the corn crop, itself, and potentially the subsequent crop. We tested adding additional inputs (starter fertilizers and plant growth regulators) at planting along with nematicides to provide a complete management system of both *M. incognita* and the corn plant by improving plant health and at the same time suppressing nematode population density. Each input was evaluated separately in a greenhouse setting and individual commercial products were tested on efficacy and/or use rate. The most effective products at both increasing plant biomass and decreasing or at least maintaining *M. incognita* population density were amalgamated into a single trial to further test their efficacy in a field setting. These products were two nematicides, Terbufos and Fluopyram, the plant growth regulator, Ascend, as an in-furrow application, and the combination of starter fertilizers (Pro-Germinator + Sure-K + Micro 500) for in field and microplot settings to determine yield effects. The fields (PBU and BARU) are infested with *M. incognita* race 3. Data were subjected to analysis of variance in SAS 9.4 using the PROC GLIMMIX procedure and means separated using Dunnett's method with $P \leq 0.10$. At PBU, *M. incognita* eggs/g of root ranged from as low as 191 with the Terbufos + Ascend + starter fertilizer package (SF) to as high as 2769 eggs/g of root with the Fluopyram + SF. However, the control had similar *M. incognita* eggs/g of root to all other treatments. Terbufos + Ascend + SF increased ($P \leq 0.1$) both plant biomass and height over the control. Yield was not significantly increased ($P \leq 0.1$) with any of the treatments. At BARU, both Terbufos + SF and Terbufos + Ascend + SF increased ($P \leq 0.1$) yield, plant vigor, height, and biomass, compared to the control. Although *M. incognita* eggs/g of root were much lower than that of PBU, significant decreases compared to the control were still seen with Terbufos alone, Terbufos + SF, and Terbufos + Fluopyram + Ascend + SF. Overall, for the 2016 growing season, the addition of SF to Terbufos increased yield by 2 bu/A and 28 bu/A for PBU and BARU, respectively, while the addition of the plant growth regulator, Ascend, to the aforementioned treatment resulted in an 11 bu/A and 34 bu/A increase, respectively. Significant yield increases were only observed at BARU. After accounting for additional input costs at BARU, we saw a net gain of $31.40/A for the Terbufos + SF treatment, and a net gain of $44.98/A for the Terbufos + Ascend + SF treatment at the market price of $3.50/bu.

EFFECTS OF CO-INOCULATION WITH *PRATYLENCHUS PENETRANS* AND *FUSARIUM OXYSPORUM* ON POTATO EMERGENCE, GROWTH AND YIELD. **Upadhaya, Arjun[1], G.P. Yan[1], A. Plaisance[1], G. Secor[1], and A. Robinson[2].** [1]North Dakota State University, Department of Plant Pathology, Fargo, ND 58108, [2]NDSU, Department of Plant Sciences, Fargo, ND 58108.

Potato emergence disorder (PED) is frequently observed in commercial potato farms in central Minnesota. The disorder has been studied for several years and the cause of the problem remains unknown. This problem occurs in sandy soils and has not been associated with nutrient imbalances, herbicide injury or a pathogen. The symptoms include poor emergence and stunted plants often in patches or areas in the field. Soil-borne nematode pathogen, *Pratylenchus penetrans*, and fungal pathogen, *Fusarium oxysporum*, are commonly present in fields with PED. It was therefore hypothesized that those pathogens may play a role in the PED as they both are important pathogens of potato. A microplot study was carried out in 2016 at the Sand Plain Research Farm in Becker, Minnesota to evaluate the effects of the inoculation of these pathogens singly and in combination on emergence, growth and yield of the potato cultivar Red Norland. *P. penetrans* (200, 800 or 2,000 nematodes per 5 kg of soil) and *F. oxysporum* (5, 10 or 20 colonized barley seeds per 5 kg soil) were either inoculated individually or co-inoculated. Emergence rate (50%) was lowest at high level (2000 nematodes + 20 fungus-colonized seeds) of co-inoculation, compared to high fungus alone (60%), high nematode alone (70%), and non-inoculated control (90%). The high level of nematode alone significantly reduced ($P < 0.05$) the plant height, shoot weight, root weight and yield by 26, 28, 42, and 29 %, respectively, when compared to non-inoculated control. High *F. oxysporum* alone reduced ($P < 0.05$) the plant height, shoot weight, root weight and yield by 21, 36, 37, and 34 %, respectively. Reduction of these same parameters ($P < 0.05$) was

26, 44, 43, and 43%, respectively, due to co-inoculation at the high level. Similarly, the medium level of pathogens alone and co-inoculation significantly reduced the plant height and yield. However, the low level of inoculum did not result in significant reduction of plant parameters. *P. penetrans* were not detected inside the tubers but were recovered from roots with characteristic brown lesions, in nematode alone and co-inoculation treatments. Stem end rot near eyes of tubers and fungal growth in wounded tuber parts were the internal symptoms in cut tubers of fungal pathogen alone and co-infection treatments. Disease incidence (22%) and severity (22%) in tubers were greater at the high co-infection level than that (incidence = 10%, severity = 13%) at the high fungus alone. Compared to non-inoculated control, wilting % in foliage was significantly higher ($P<0.05$) at the high co-infection level (49%) than the high nematode only (29%) and high fungus only (37%). Similarly, wilting % at the medium and low co-infection levels was higher than fungus only. From our preliminary results, presence of both pathogens at the high level can cause more negative effects on potato emergence, growth, yield, and disease incidence and severity compared to presence of only one pathogen at the same level.

IDENTIFICATION OF A PANEL OF EFFECTOR GENES FOR *PRATYLENCHUS PENETRANS*. **Vieira, Paulo[1,2], T. Maier[3], S. Eves-van den Akker[4], I.A. Zasada[5], T. Baum[3], J.D. Eisenback[1] and K. Kamo[2].** [1]Virginia Tech, Dept. of Plant Pathology, Physiology, and Weed Science, Blacksburg, Virginia, [2]USDA ARS, Floral and Nursery Plants Research Unit, BARC, [3]Department of Plant Pathology, Iowa State University, Ames, Iowa, [4]Division of Plant Sciences, College of Life Sciences, University of Dundee, Dundee, UK, [5]USDA ARS, Horticultural Crops Research Laboratory, Corvallis, Oregon.

Root lesion nematodes (RLN), namely *Pratylenchus* spp., are economically important pathogens that inflict damage and loss of yield to a wide range of crops. Like other plant-parasitic nematodes, RLN require close association with their host to gain access to nutrients. The successful infection of plant-parasitic nematodes relies on the secretion of a repertoire of proteins (often called effectors) with diverse parasitism-related functions. So far, a reduced number of effectors have been validated or characterized for RLN. One of the aims of this study was the identification of a panel of nematode effector genes for *P. penetrans*. Here we compare two different sets of transcripts generated for *P. penetrans* collected directly from the nematode esophageal glands and sequences transcriptionally active during plant interaction. In order to determine if *P. penetrans* genes represent valid candidate effectors, *in situ* hybridization assays were performed. In total, 22 genes were specifically localized within the esophageal glands of the nematode, some homologous to known effector genes of other plant-parasitic nematodes (e.g. cell-wall degrading enzymes), while others with unknown annotation and specific to RLN. We performed RT-qPCR analyses to highlight the dynamic expression of *P. penetrans* effector genes during plant infection. A discriminatory motif in the promoter region was also found for a significant number of effector candidate genes. The importance of some *P. penetrans* effector genes was studied by *in planta* RNA interference (RNAi) assays using stable soybean hairy root lines. This constitutes the first set of candidate effectors validated for *P. penetrans* and suggests that *P. penetrans* has evolved and relies on its own set of secreted proteins to be a successful parasite.

EFFECTIVE TERMINATION METHODS OF BRASSICA COVER CROPS FOR SUPPRESSION OF PLANT-PARASITIC NEMATODES WHILE ENHANCING SOIL HEALTH. **Waisen, Philip**, **K.-H. Wang, Z. Cheng** and **B.S. Sipes**. Department of Plant and Environmental Protection Sciences, University of Hawaii, Maile Way, Honolulu HI 96822.

Brassica cover crops such as brown mustard (*Brassica juncea*) and oil radish (*Raphanus sativus*) are popular for soil and plant health management due to their green manure, nutrient scavenging, and weed suppressive properties. They also biosynthesize glucosinolates that can be converted into isothiocyanates by endogenous myrosinase enzymes upon tissue maceration, thus serving as good biofumigant cover crops against plant-parasitic nematodes (PPNs). However, merely incorporating brassica tissues into the soil did not suppress PPNs effectively in a preliminary trial. Objectives of this study were to determine cover crop termination methods that could 1) maximize biofumigation effects of mustard and oil radish against root-knot (*Meloidogyne* spp.) and reniform (*Rotylenchulus reniformis*) nematodes; and 2) improve soil and plant health of cash crops. A field trial was initiated in a field heavily infested with PPNs in November 2016 where mustard and oil radish were grown for 5 weeks and terminated by 1) maceration (M) using a line trimmer followed by tilling (T) or M+T; 2) M+T followed by tarping with black plastic (BP) or M+T+BP; and 3) no-till (NT) cover cropping followed by chopping the cover crop at the soil line and covering the residues with a woven weed mat. Bare ground (BG) was included as a control. Experiment was arranged in randomized complete block design with 4 replications. Since glucose is another by-product of the glucosinolate hydrolysis, soil from each plot was collected right before, 1 hour and 7 days after cover crop termination to assay for myrosinase activities using glucose analysis. A zucchini (*Cucumis pepo*) crop was grown thereafter. All oil radish regardless of termination treatments and mustard in M+T+BP treatment had higher myrosinase activities than BG ($P \leq 0.05$) based on glucose concentration. Nematode assays performed over a 2-month zucchini cropping period revealed that both cover crops when terminated by M+T+BP reduced *Meloidogyne* spp. and root-gall index (RGI), whereas only mustard terminated with M+T+BP suppressed *R. reniformis* compared to BG ($P \leq 0.05$). Oil radish in M+T also reduced RGI compared to BG ($P \leq 0.05$). The biofumigation effect against PPNs did not compromise beneficial nematodes in the soil as

abundances of free-living nematodes were not different among treatments 2 months after zucchini planting. However, mustard in M+T+BP increased nematode diversity compared to BG ($P \leq 0.05$). As a consequence of effective suppression against both key PPNs in this soil, zucchini chlorophyll content and canopy width were also higher in M+T+BP compared to other treatments ($P \leq 0.05$). It is concluded that brassica cover crops especially mustard which is susceptible to many *Meloidogyne* spp. was more effective in suppressing PPNs in the field when the cover crop was macerated and soil incorporated, followed by tarping with a black plastic to slow down the volatiles from escaping into the atmosphere.

NEMATICIDE EFFECTS ON NON-TARGET NEMATODE POPULATIONS IN BERMUDAGRASS. **Waldo, Benjamin, W.T. Crow, Z.J. Grabau, and T.M. Mengistu.** Entomology and Nematology Dept., P.O. Box 110620, Building 970, Natural Area Dr. University of Florida, Gainesville, FL 32611-0210.

Environmental impacts of chemical management practices of pests and pathogens are important topics. In turfgrass systems, nematicides are a valuable tool for managing plant-parasitic nematode populations, but few studies have examined nematicide effects on non-target nematodes. Similar pesticide treatment studies observed shifts in soil communities to disturbed environments with a reduction of high cp value nematodes. Our study evaluated effects of turfgrass nematicide formulations of abamectin (Divanem SC), fluopyram (Indemnify), furfural (MultiGuard Protect EC), and fluensulfone (Nimitz Pro G) on non-target nematode populations. A randomized block design was used with five reps of four nematicide treatments and an untreated control. Plots were 6 $m^2$ with 0.6 m untreated borders between adjacent plots. Data were collected from 1.5 $m^2$ subplots located in the center of the treatment plots. Nematicides were applied at labeled rates every four weeks as a summer treatment program from 7 June to 30 August, 2016 at the University of Florida Plant Science Research and Education Center in Citra, Florida. Samples were collected before treatment and two days, two weeks, and eight weeks after the final treatment for community analysis. Data from each nematicide treatment were compared to the untreated at each sample date using analysis of covariance with initial population counts serving as the covariate. Abamectin treated plots showed a significant reduction in fungivore nematodes in samples taken two days, two weeks, and eight weeks after final treatment. Fluopyram treated plots had significant reduction of bacterivores, fungivores, and omnivores two days, two weeks, and eight weeks after the final treatment. Furfural treated plots showed a reduction in omnivore nematodes two days and two weeks after final treatment. Fluensulfone treated plots showed a reduction in bacterivores two days post final treatment and a reduction in fungivores eight weeks after final treatment. Community analysis indicated a shift toward a disturbed environment in treated plots. Significant reduction in MI, MI2-5, and ΣMI indices were observed two days and two weeks after final treatment for most nematicides. Fluopyram treated plots had significant reduction after eight weeks for all three indices mentioned, but the majority of plots showed recovery to non-significant differences from the untreated control. Faunal analysis revealed a shift of data points from a structured undisturbed environment in quadrant C toward a more stressed and disturbed environment in quadrants A and D. The first year results of our study suggest nematicide treatments can significantly influence nematode community structure.

RESPONSES OF HETERODERA GLYCINES AND MELOIDOGYNE INCOGNITA INFECTIVE JUVENILES TO ROOT TISSUES, ROOT EXUDATES, AND ROOT EXTRACTS FROM 3 PLANT SPECIES. **Wang, Congli[1,2], S.T. Rogers[2], and E.P. Masler[2]**. [1]Key Laboratory of Mollisols Agroecology, Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences, Harbin 150081, China, [2]USDA-ARS Mycology and Nematology Genetic Diversity and Biology Laboratory, Beltsville, MD, 20705.

The infective juvenile (J2) stage of endoparasitic plant nematodes utilizes cues to chemical signals, released from roots, to localize and infect hosts. Recently[3], J2 of *Heterodera glycines* (soybean cyst nematode, SCN; reared on soybean, *Glycine max*) were found to be attracted to crude whole root extract slurries of marigold (*Tagetes patula*), pepper (*Capsicum annuum*) and soybean, whereas *Meloidogyne incognita* J2 (root-knot nematode, RKN; reared on pepper) were either not attracted or were repelled by the same extracts. These J2 behaviors are curious given the rather narrow host range of SCN and the somewhat greater host range of RKN. Consequently, we have expanded work on plant signals to include root tips, root exudates, and root extracts, three sources commonly used in phytoparasitic nematode chemotaxis studies. Root tips from marigold, pepper, and soybean each attracted RKN, but SCN was attracted only to soybean. In contrast, root exudates, prepared by briefly soaking marigold, pepper, or soybean seedlings in tap water, were attractive to SCN but were repellent to RKN, similar to the response to whole root slurries. Root extracts (fresh or rehydrated) had the same effect as exudates, with SCN universally attracted and RKN universally repelled. We have begun fractionation of extracts and exudates in an effort to discover biochemical differences among sample sources (plant species; extracts, exudates) and to facilitate characterizing the components responsible for attraction and repulsion. In initial efforts, we used a $C_{18}$ reverse phase stepwise elution method to collect polar and non-polar fractions. All *G. max* fractions from each source were attractive to SCN, indicating the presence of at least two chemically different attractants in root extracts and in exudates from soybean. SCN were also attracted to the polar and non-polar fractions from *C. annuum* and *T. patula* exudates. However, only the polar fractions from pepper and marigold extracts were attractive to SCN, indicating the existence of biochemical differences between extract and exudate that are not apparent in non-fractionated samples. Preliminary tests with RKN show that soybean exudate and extract polar fractions are repulsive but that non-polar fractions have no effect. RKN tests with pepper and marigold are ongoing.

Differences among extract and exudate components, contrasts between root tips and the various root samples relative to J2 behaviors, and appearance of toxicity to J2 in selected non-fractionated and fractionated samples are discussed.

FACTORING NEMATODE SOIL HEALTH INDICATORS INTO PLANT AVAILABLE NITROGEN MINERALIZATION RATE FOR COVER CROPS. **Wang, Koon-Hui[1], S. Ching[1], J. Marquez[1], P. Waisen[1], T. Radovich[2], N. Andrew[3], and D. Sullivan[3].** [1]Dept. Plant and Environmental Protection Sciences, [2]Department of Tropical Plant and Soil Sciences, University of Hawaii at Manoa, Honolulu, HI; [3]Department of Crop and Soil Science, Oregon State University, Corvallis, OR.

Cover crops are often grown as green manure because the nutrients they release help reduce fertilizer inputs. This research examines nitrogen (N) accumulated in cover crop tissue that can be released into plant available N (PAN). Scientists had developed cover crop calculators to estimate percentage of PAN that can be made available over time after termination of a cover crop based on tissue N content and cover crop dry biomass. Vigil-Kissel's equation (1991) has been commonly used in the Pacific Northwest of the U.S. to estimate PAN mineralization rates (PAN%). However, this equation tends to under estimate PAN% of eight different soils tested in Hawaii at variable levels. While PAN% could be affected by climate, soil types, and farming practices, the objectives of this project were to determine if soil health conditions affect PAN% and if so, to what extent. Soil health conditions of six soils collected from different islands in Hawaii encompassed andisols, inceptisols, mollisols, and oxisols, ranging from 20 to 853 m in elevations were categorized based on Enrichment (EI) and Structure indices (SI) of nematode fauna analysis. All soils tested were incorporated with sunn hemp (*Crotalaria juncea*) tissues at 1% (dry weight equivalent) amendment rate and incubated at 23°C. PAN% were estimated at 28 and 70 days after soil incubation as described by Sullivan and Andrew (2012). Canonical analyses (CCA) were used to deduce the relationship between abundance of five nematode trophic groups, with PAN%, N%, richness, diversity, EI, SI, and CI (Channel index). When analyzing soil with a nematode community dominated by high EI but low SI, CCA indicated that PAN% was positively correlated with EI and abundance of bacterivores, and negatively correlated with SI and CI (first two axes explained 99.6% of the cumulative variance). However, when analyzing soil with nematode communities with a wider distribution of EI-SI trajectories, PAN% was positively correlated with SI, CI and abundance of omnivores, but negatively correlated with EI (first two axes explained 96.7% of the cumulative variance). Soil health conditions will most likely improve over time as the practice of cover crop rotation continues. Thus, PAN% should be adjusted as EI or SI increases over time. This research present the models to adjust PAN% based on SI and EI of the soil.

INFORMATION TRANSFER: ALMOST 20 YEARS LATER. **Westerdahl, Becky, and E.P. Caswell-Chen.** Department of Entomology and Nematology, University of California, Davis, CA 95616.

In 1998, in an ASS-CSAA-SSSA Monograph on *Plant Nematode Interactions,* the authors presented a chapter on ''Information Transfer'' in which we summarized the state of digital communication technology at that point in time. We predicted our offering would be out of date long before any of the other chapters. We will now offer a perspective on what has changed and what has remained the same since our 1998 publication. Emphasis will be on information delivery via digital technology to Cooperative Extension clientele, particularly via websites and on-site presentations. Although much has changed as we have hurtled through the intervening years, the following sentences we wrote then seem to still be applicable today: ''The optimal time to make a point to a student or client is at the time when the information is needed. With the development of the personal computer, the 'teachable moment' may be expanded in ways that were not previously possible. In the past, information transfer was relatively simple and accomplished via two primary means-verbal and written. The primary means for information transfer remain the same, but the technological means for effecting transfer are new. All of these new information technologies will advance the abilities of agricultural professionals to identify and monitor nematode problems in the field, research a wide range of possible solutions, and apply appropriate management tactics in a site-specific manner.''

AN EXAMPLE FOR THE REGULATORY FRAMEWORK FOR RELEASE OF CULTIVARS WITH RESISTANCE TO PLANT-PARASITIC NEMATODES. **Westphal, Andreas.** Department of Nematology, University of California Riverside, Parlier, CA 93648.

Plant-parasitic nematodes are yield-reducing pests that put sustainable crop production at risk. The use of host plant resistance is widely recognized as an efficient management tool to reduce the negative impacts of parasitic nematodes. This information has been used in various ways. For example, a comprehensive regulatory framework is in place in Germany to recognize the importance and possible implementation of this valuable trait. In Europe, mutual legislation, or country legislation impacts the stipulations of such guidelines. The most comprehensive legislation probably is implemented for potato cyst nematodes (PCN), *Globodera pallida* and *G. rostochiensis*. The resort research institutes are conducting the testing necessary to fulfill the legal requirements. European legislation mandates how to manage PCN by giving detailed and comprehensive instructions on how to sample fields, how to extract the nematodes, and how to test candidate potato cultivars for host response to PCN. Results from these Europe-wide comparable tests are shared among the EU-member countries and included in respective official publications that state the binding classification of potato cultivars. Probably, the foremost market-driven practice is the process for the release of *Brassica* lines for cover crops. Because of the importance of cover cropping using *Brassica* cultivars in sugar beet rotations for

suppression of *Heterodera schachtii*, mustard and oil seed radish cultivars are tested according to a clearly defined testing protocol. This system is in place since inception of this Brassica cover crop use, and provides the marketing framework for *Brassica* lines. Testing systems are less regulated when they support export potential of small grain lines because of resistance to *H. avenae* and *H. filipjevi*. At the same time, uniform testing systems are being developed since resistant traits gain importance in the marketing of cultivars, as is the case for *H. schachtii*-tolerant sugar beet cultivars. In summary, this comprehensive framework incentivizes the development of cultivars with increasing levels of resistance and tolerance to plant-parasitic nematodes.

USE OF NOVEL CHEMISTRIES FOR THE SUPPRESSION OF *PRATYLENCHUS* VULNUS IN NURSERY SETTINGS. **Westphal, Andreas, T.R. Buzo, and Z.T.Z. Maung.** Department of Nematology, University of California Riverside, Parlier, CA 93648.

In California, the production of healthy and non-infested planting stock of tree and vine crops is legislatively regulated. Mandatory procedures include sampling at harvest or by following strictly defined treatment protocols to ensure nematode free rootstocks. *Pratylenchus vulnus* is important in these considerations. Treatment options were severely reduced after phase-out of methyl bromide, and use of 1,3-D-containing materials is restricted by township caps. Alternative soil treatments were examined in microplot experiments. On 1 October 2015, four low-volume materials, one including the active ingredient Vellozine, and three biocidal of different modes of action, high-volume materials were applied to a sandy loam soil in water drenches (150 L/m$^2$) and compared to the controls of a water drench or a Telone II fumigation. On 22 November 2015, live plant-parasitic nematodes were detected in the soil profile and pre-germinated seeds of 'Nemaguard' peach were planted to the microplots. In the following growing season, infection rates of *P. vulnus* in the peach seedlings were assessed at specific time intervals. Initially, root penetration and later populations after mist extraction were determined. After 16 months of cultivation, plants grown in plots with large-volume biocide treatments, and Vellozine had similar trunk diameters as the Telone II control, significantly thicker than the water control. *P. vulnus* was virtually absent from the Telone II and the Vellozine treatments. Other treatments with low-volume materials resulted in the presence of some nematodes and small trunk diameters similar as the water control. In second microplot experiment, selected treatments were applied in the same way to a sandy soil or a sandy loam soil on 4 March 2016. On 20 April 2016, clonal 'Nemaguard' plugs were planted into the soils. In that test, root examinations suggested that biocide treatments were more effective than non-fumigant materials but final evaluations are still outstanding. Application methods, e.g. continuous drench versus pulsed applications, and the effects of soil texture composition will need further investigation, but initial results indicate some promise that novel chemistries will provide alternatives to soil fumigation for nursery applications.

IDENTIFICATION OF MOLECULAR BIOMARKERS ASSOCIATED WITH RENIFORM NEMATODE RESISTANCE IN SOYBEAN. **Wilkes, Juliet[1], P. Agudelo[1], B. Fallen[2], C. Saski[1] and J. Mueller[3].** [1]Biosystems Research Complex, Clemson University, Clemson, South Carolina 29634, [2]Pee Dee Research and Education Center, 2200 Pocket Rd., Florence, South Carolina 29506, [3]Edisto Research and Education Center, 64 Research Rd., Blackville, South Carolina 29817.

Reniform nematode (*Rotylenchulus reniformis*) is a yield-limiting pathogen of soybean (*Glycine max*) in the Southeastern region of the United States. Several studies have identified soybean germplasm with resistance to reniform nematode. However, only a few studies have explored the soybean genome for quantitative trait loci (QTL) linked to reniform nematode resistance. Our objective for this study was to identify high resolution single-nucleotide polymorphism (SNP) biomarkers that correlate with reniform nematode resistance in soybean using genotyping-by-sequencing (GBS). A set of 250 recombinant inbred lines developed from a cross between cultivars 'Forrest' and 'Williams 82' was utilized to correlate reduced nematode reproduction to SNP markers, localizing specific QTL regions. The phenotype was determined by growing three replicates of each line in eight-centimeter diameter cups in a growth room maintained at 28°C. Each plant was inoculated with 2000 vermiform reniform nematodes and populations quantified two months after inoculation. Leaf tissue was collected from each line for genotyping. Reduced representation next-generation sequencing techniques were employed to achieve high-density genetic screening through GBS. DNA from each line was digested using specific restriction enzymes *MseI* and *PstI* to prepare gene libraries, then sequenced on the Illumina HiSeq platform and analyzed using *Stacks* software program to generate population genomic summary statistics. We report SNP markers that correlate to the resistant phenotype observed in the developed lines. The characterized genetic markers can be used by soybean breeders in marker assisted selection to enhance their efforts in selecting and employing lines with known resistance to reniform nematode.

LEARNING ABOUT LEARNING: EDUCATING ENTOMOPATHOGENIC NEMATODES FOR BIOLOGICAL CONTROL. **Willett, Denis S.[1,3], L.L. Stelinski[1], L.W. Duncan[1], D.I. Shapiro-Ilan[2], H.T. Alborn[3].** [1]Citrus Research and Education Center, University of Florida, 700 Experiment Station Road, Lake Alfred, FL 33850 [2]Southeastern Fruit and Nut Research Lab, USDA-ARS, 21 Dunbar Road, Byron GA 31008 [3]Center for Medical, Agricultural, and Veterinary Entomology, USDA-ARS, 1600 SW 23rd Drive, Gainesville, FL 32608.

Despite having a limited number of neurons, nematodes demonstrate a remarkable capacity for behavioral plasticity. Entomopathogenic nematode infective juveniles, for example, alter behavior based on past experiences and chemically

mediated communication with other belowground organisms. Here, we discuss the ramifications of such behavioral plasticity in the context of belowground multi-trophic interactions. We highlight recent work into the ecology of such interactions and show how knowledge of these dynamics can be used to enhance biological control of insect pests.

THE CAPACITY OF THE SAPOPHYTIC FUNGUS FUSARIUM SOLANI TO AFFECT THE POPULATION DYNAMICS AND INSECTICIDAL EFFICIENCY OF STEINERNEMA DIAPREPESI. **Wu, Sheng-Yen[1], F.E. El-Borai [1,2], and L.W. Duncan[1].** [1]University of Florida, Citrus Research and Education Center, 700 Experiment Station Rd., Lake Alfred, FL 33850, USA, [2]Plant Protection Department, Faculty of Agriculture, EL-Zagazig University, Egypt.

In a field survey, the saprophytic fungus, *Fusarium solani* was isolated from 42% of *Galleria mellonella* sentinel larval cadavers that did not have evidence of entomopathogenic nematode (EPN) reproduction. Based on published reports that *F. solani* is entomopathogenic, we tested the hypothesis that *F. solani* competes with EPNs for insect prey, thereby reducing EPN efficacy in the field. Conidia of *F. solani* and infective juvenile *Steinernema diaprepesi,* alone or in combination, were added to soil microcosms containing sentinel larvae of the weevil *Diaprepes abbreviatus*. Significantly more weevils were killed (83%) in the concomitant species treatment compared to treatments with only the EPN (58%) or the fungus (0%). Although *F. solani* conidia increased the number of cadavers supporting nematode reproduction, the EPN fecundity per cadaver did not differ in the absence or presence of the fungus. We employed two-choice, t-tube assays to determine whether *F. solani* might recruit EPNs and thereby increase the availability of insect cadavers. When given a choice, *S. diaprepesi* migrated in greater numbers toward the side of tubes containing agar plugs with *F. solani* mycelia and conidia compared to the side with only agar plugs. However, this tendency attenuated in proportion to the complexity (addition of insects, use of raw rather than sterile soil, etc.) of habitat. Our data support the plausibility that *F. solani* increases the effectiveness of *S. diaprepesi* in order to exploit the resources in the cadaver. Ongoing experiments seek to identify volatile or soluble putative EPN attractant compounds produced by *F. solani*.

ADVANCES IN THE DEVELOPMENT OF COTTON GERMPLASM RESISTANT TO ROOT-KNOT AND RENIFORM NEMATODES – A COTTON MAS SUCCESS STORY. **Wubben, Martin J.[1], J.C. McCarty Jr.[1], J.N. Jenkins[1], F.E. Callahan[1], R.W. Hayes[1], and D. Deng[1].** [1]USDA-ARS, Crop Science Research Laboratory, 810 Highway 12 East, Mississippi State, MS 39762.

The southern root-knot nematode (RKN), *Meloidogyne incognita* (Kofoid and White), and reniform nematode (RN), *Rotylenchulus reniformis* (Linford and Oliveria), are perennial causes of significant yield loss in Upland cotton (*Gossypium hirsutum* L.) production areas of the United States. Annual cotton yield losses to both species can vary significantly between fields and between states but usually exceed a total of $100 million/year. The laborious and time-consuming nature of nematode resistance screening, coupled with the high level of experimental variability inherent to resistance phenotyping, have stood for decades as nearly insurmountable obstacles in the development of germplasm having both nematode resistance and superior agronomic traits. Only with the advent of new genomic and bioinformatic tools combined with large, robust mapping populations has significant progress been possible in making RKN and RN resistance available to cotton producers. The discovery of molecular markers linked to RKN resistance quantitative trait loci (QTL) on chromosomes 11 and 14 of lines derived from the highly resistant Auburn 623 RNR germplasm was a tremendous breakthrough that has allowed this resistance to be incorporated into commercially available elite cultivars. Likewise, genetic mapping studies of the highly RN-resistant wild *G. barbadense* L. accession GB-713 and subsequent genetic dissection of the resistance QTLs on chromosomes 18 and 21 will facilitate the deployment of RN resistance throughout cotton growing regions of the U.S. Furthermore, the availability of RKN and RN resistance markers has recently allowed the release of six germplasm lines by our laboratory that harbor resistance to both species. This symposium talk will present an overview of the history of RKN and RN resistance breeding in cotton and discuss how the aforementioned discoveries made by USDA-ARS and university researchers have combined to make cotton nematode resistance breeding an excellent example of a marker-assisted-selection (MAS) breeding program.

NEMATICIDE EVALUATIONS FOR THE MANAGEMENT OF *MELOIDOGYNE INCOGNITA* AND *ROTYLENCHULUS RENIFORMIS* IN COTTON. **Xiang, Ni, and K.S. Lawrence.** Department of Entomology and Plant Pathology, Auburn University, Auburn, AL 36849.

The objective of this work was to evaluate the effect of Fluazaindolizine in-furrow applications for the management of *M. incognita* and *R. reniformis* on cotton production in infested fields in central and north Alabama. Fluazaindolizine, a new nematicide developed by DuPont. The nematicide was applied at planting in furrow spray application and in some treatments an added foliar spray application was applied at the 6 to 8 leaf stage. Treatments of the Fluazaindolizine were: 1) 1.17 l/ha in-furrow, 2) 2.34 l/ha in-furrow, 3) 1.17 l/ha in-furrow +2.34 l/ha foliar spray, 4) 1.17 l/ha in-furrow + 1.24 l/ha foliar spray of Vydate C-LV, 5) 2.34 l/ha in-furrow + 1.24 l/ha foliar spray of Vydate C-LV, 6) 1.24 l/ha foliar spray of Vydate C-LV, and 7) untreated control. The field trials were placed in RCBD with five replications and were irrigated. Initial population densities were average at 3500 vermiform life stages per 150 $cm^3$ of soil for *R. reniformis* and 160 J2 per 150 $cm^3$ of soil for *M. incognita*. Results indicated that plant stand or plant survival was similar between all nematicide treatments and the

untreated control in both *R. reniformis* and *M. incognita* infested fields and no phytotoxicity was observed with the nematicides applications. The 1.17 l/ha and 2.34 l/ha in-furrow, 1.17 l/ha in-furrow +2.34 l/ha foliar spray, 2.34 l/ha in-furrow + 1.24 l/ha foliar spray of Vydate C-LV, and 1.24 l/ha foliar spray of Vydate C-LV significantly reduced the number of *M. incognita* eggs/g of root as compared to untreated control at 40 day after planting (DAP) ($P \leq 0.10$). The 2.34 l/ha in-furrow, 1.17 l/ha in-furrow + 2.34 l/ha foliar spray, and 2.34 l/ha in-furrow + 1.24 l/ha foliar spray of Vydate C-LV significantly reduced the number of *R. reniformis* eggs/g of root on cotton as compared to untreated control at 40 DAP ($P \leq 0.10$). Additionally seed cotton yield was increased. The application of 2.34 l/ha in-furrow also significantly enhanced seed cotton yield by an average of 31% and 62% as compared to untreated control in both *M. incognita* and *R. reniformis* infested fields, respectively ($P \leq 0.10$). Linear regressions of the cotton yield as influence by nematode populations at 40 DAP were significant indicating *M. incognita* and *R. reniformis* populations negatively influenced cotton yield.

*OSCHEIUS WISCONSINENSIS* N. SP. (NEMATODA: RHABDITIDAE), A POTENTIAL ENTOMOPATHOGENIC NEMATODE FROM THE MARSHLANDS OF WISCONSIN. **Ye, Weimin[1], S. Foye[2] and S. Steffan[2,3].** [1]Nematode Assay Section, Agronomic Division, North Carolina Department of Agriculture & Consumer Services, Raleigh, NC 27607. [2]Russell Laboratories, Department of Entomology, University of Wisconsin-Madison, 1630 Linden Drive, Madison, WI 53706, USA. [3]United States Department of Agriculture, Agricultural Research Service, Madison, WI 53706, USA.

*Oscheius wisconsinensis* n. sp. (Rhabditidae) was recovered through the *Galleria* bait method from a wild cranberry marsh in Jackson County, Wisconsin, USA. Morphological studies with light microscopy and scanning electron microscopy, as well as molecular analyses of the near-full-length small subunit rDNA gene (SSU), D2/D3 expansion segments of the large subunit rDNA gene (LSU), internal transcribed spacer (ITS), and mitochondrial cytochrome oxidase subunit 1 (CO1) genes revealed this as a new species, described herein as *Oscheius wisconsinensis* n. sp. The new species is characterized by its unique DNA sequences; hermaphroditic reproduction; male absent. *Oscheius wisconsinensis* n. sp. belongs to the *dolichura*-group. A *Bacillus*-like bacteria appears to be associated with this nematode based on our microscopic and SEM observations. Preliminary tests revealed that this nematode is capable of infecting Blattodea, Coleoptera, and two families of Lepidoptera under laboratory conditions; therefore, it has potential as a biological control agent.

BEAUVERIA BASSIANA REDUCES EGG HATCH OF ROOT KNOT NEMATODE AND INCREASES DEATH OF JUVENILES. **Yerukala, Shalini, E.C. Bernard, and B.H. Ownley.** 2505 E.J. Chapman Drive, 370 Plant Biotechnology, Department of Entomology and Plant Pathology, University of Tennessee, Knoxville, TN 37996-4560.

*Beauveria bassiana* has been reported as an endophyte of several plant species. It has also been shown to protect host plants against insect pests and plant pathogens, but much less is known about its activity against plant-parasitic nematodes. To determine the effect of *B. bassiana* isolate 11-98 on egg hatch of *Meloidogyne incognita, in vitro* bioassays were conducted. Five separate experiments were performed with different ratios of *B. bassiana* spores, root knot nematode eggs, and water, and the mixtures were incubated for short and long time periods (41 hours and either 169.5 or 262.5 hours). The calculated ratios of total fungal spores to total nematode eggs per volume (ml) of water in bioassays were the following: 0.4, 1.0, 1.5, 2.3, and 2.9. Nematode eggs were extracted from galled tomato roots in tap water with sieving and decanting. A suspension of eggs was diluted to 150 eggs per 100 microliters of tap water, and various rates were prepared for the bioassays. *Beauveria bassiana* was grown on potato dextrose agar (PDA) for approximately 3 weeks at room temperature. Dry spores of *B. bassiana* were collected from PDA plates and concentrations of 265 and 2650 spores per 100 microliter of sterile deionized water were prepared, and used in these assays. Viable population counts were confirmed with standard dilution plating onto PDA. Controls were included in each bioassay and consisted of nematode eggs suspended in tap water mixed with sterile distilled water with no fungal spores. There were 16 to 24 replications per bioassay. Data were analyzed for significance with SAS 9.4. Bioassays were performed in 24-well tissue culture plates or quadrant Petri dishes. After incubation for 41 hours, egg hatch was significantly reduced at the three highest ratios of fungal spores to nematode eggs per milliliter of water, compared to controls. There were no significant differences in egg hatch of the two lowest ratios, compared to controls. At longer incubation times, there were significant differences in the numbers of dead nematodes in treated vs. control. In all cases, no dead nematodes were observed in the controls. For the highest ratios of fungal spores to nematode eggs per volume water, and a long incubation time of 169.5 hours, there was a significant reduction in egg hatch for the highest ratio compared to control. A reduction in egg hatch was also observed for one of the lower ratios compared to control, with a longer incubation time (262.5 hours). The results suggest that *Beauveria* has an inhibitory effect on egg hatching; however, effectiveness varies with concentration of spores and incubation time. The presence of dead nematodes in treatments with *Beauveria* spores and the lack of dead nematodes in controls suggests that *Beauveria* has nematicidal characteristics, and thus, has potential for development of control strategies.

NEMATODE SUPPRESSIVE EFFECTS OF VERMICOMPOST TEAS PREPARED FROM BAMBOO AND KUDZU VS THAT FROM VEGETABLE FOOD WASTE. **You, Xiaodong[1,2], K.-H. Wang[1], and M. Tojo[2].** [1]Deparment of Plant and Environmental Protection Sciences, University of Hawaii at Manoa, Honolulu, HI; [2]Graduate School of Life and Environmental Sciences, Osaka Prefecture University, Sakai, Osaka 599-8531, Japan.

Invasive plant species such as bamboo (*Phyllostachys edulis*) and kudzu (*Pueraria lobata*) are extremely destructive to ecosystems in Japan. Researchers are finding uses for these invasive plants. A laboratory assay was established to examine the effects of vermicompost tea (VCT) prepared from bamboo and kudzu (VBK) and that prepared from vegetable food waste (VFW) on the hatching of *Meloidogyne incognita* and *Rotylenchulus reniformis*. Water was used as a control. Each treatment had 4 replications. Both VCTs reduced *M. incognita* egg hatch by 54.4% compared to the control ($P \leq 0.05$). No significant difference between VBK and VFW was observed. VCT from VBK and VFW reduced *R. reniformis* egg hatch by 79.5% and 45.5% respectively compared to the control ($P \leq 0.05$). In a second laboratory assay, mortality of $J_2$ of *M. incognita* immersed in VCT from VFW (22.0%) was two times higher than that from VBK (11.3%), and both VCTs had higher $J_2$ mortality than the control ($P \leq 0.05$). Two greenhouse experiments were then conducted to examine the potential of these VCTs to induce host plant resistance against *M. incognita* and *R. reniformis* infection on cucumber (*Cucumis sativus*) and cowpea (*Vigna unguiculata*), respectively using split-root assays. Roots were split into two conjoined pots. One side of the root was drenched with water or VCT from VBK or VFW 3 days prior to nematode inoculation. 1 week after *M. incognita* inoculation, or 3 weeks after *R. reniformis* inoculation, roots from the nematode inoculated side were stained with Acid Fuchsin to check for nematode infection rate. VCT from VBK reduced number of *M. incognita* per gram of root by 69.0%, but VCT from VFW had no significant effect compared to the control. A field experiment was conducted to compare nematode suppressive effects of VCT from VBK or VFW using cowpea as a bioassay crop in a *M. incognita* and *R. reniformis* infested field. Cowpea plants were drenched with VCT from VBK or VFW, or water weekly over a 2-month period. Each experimental plot was $1 \times 3$ $m^2$ with 4 replications. The experiment was repeated once. Soil nematode population densities were monitored at pre-plant, 1, and 2 months after planting. Plant biomass and root gall index were recorded at the termination of each trial. These experiments showed that VCT from VBK performed similarly to VFM in suppressing *M. incognita* egg hatch but better than VFW in suppressing *R. reniformis* egg hatch. Although VCT from VBK was not as effective as VFM in suppressing mobility of *M. incognita*, it was more effective in inducing host plant resistance. Research is in progress, but results collected so far show promising use of invasive bamboo and kudzu through vermicomposting against the plant-parasitic nematodes.

CYST-FORMING NEMATODES IN HETERODERINAE IN CANADA. **Yu, Qing.** Agriculture and Agri-Food Canada, Ottawa Research and Development Centre, Ottawa, ON, K1A 0C6, Canada.

Cyst-forming nematodes of the Heteroderinae subfamily are diverse in Canada; and are represented by the genera *Bidera, Cactodera, Doliochodera, Globodera, Heterodera,* and *Punctodera*. These include some of the most economical important pests such as the soybean cyst nematode, *H. glycines*, and the potato cyst nematodes, *G. rostochineses* and *G. pallida*. Recent new discoveries of the potato cyst nematode *G. rostochinese* in 2005, and the soybean cyst nematode in 2013 in Quebec as well as the most recent carrot cyst nematode (*Heterodera carotae*) and fig cyst nematode (*H. fici*) in Ontario in 2016 are worrisome. This paper will present an overview of the biodiversity, taxonomy and an up to date distribution of the cyst nematodes from the subfamily in Canada.