#### ABSTRACTS

CURRENT STATUS OF THE BLOAT NEMATODE ON GARLIC IN NEW YORK. Abawi<sup>1</sup>, George S., K. Moktan<sup>1</sup>, C. Stewart<sup>2</sup>, R. Hadad<sup>3</sup>, and C. Hoepting<sup>4</sup>. <sup>1</sup>Department of Plant Pathology and Plant-Microbe Biology, NYSAES, Cornell University, Geneva, NY 14456; <sup>2</sup>CCE, Troy, NY 12180; and <sup>3</sup>CCE, Lockport, NY 14094; <sup>4</sup>CCE, Albion, NY 14411.

A destructive outbreak of the stem and bulb (bloat) nematode (*Ditylenchus dipsaci*) on garlic was first observed in a commercial field in western New York in June 2010. A follow-up survey demonstrated the widespread occurrence of the bloat nematode on garlic grown throughout the state and it was recovered from samples received from 17 counties and at populations as high as 3,609/g garlic tissues. Since then, over 300 garlic samples were processed in our laboratory and the bloat nematode was recovered from approximately 30% of the samples. Damage by this outbreak had significantly impacted garlic production in New York; with 100% yield losses occurring in some cases. Severely infected plants exhibit stunting; yellowing and collapse of outer leaves, and such plants may eventually die. Individual cloves or the entire bulb of infected plants initially exhibit light discoloration, later become dark brown in color, shrunken, soft, light in weight, and at later stages show cracks in the basal plate. Decay symptoms are usually developed on such cloves and bulbs due to the involvement of various saprophytic soil organisms. During 2011 and 2012, five workshops on the biology and management of the bloat nematode and general garlic production were offered and attended by >200 growers. A new project by the Specialty Crop Program through the NYS Dept. of Agriculture and Markets will subsidize the bloat nematode diagnostics and outreach on appropriate garlic and soil sampling protocols, interpretation of the test results obtained, and the implementation of appropriate management options against this nematode for both conventional and organic garlic growers in New York. The latter include the use of nematode-free planting seeds, planting in bloat nematode-free or treated soil, use of appropriate crop rotations (out of Allium species and other known hosts to the garlic and onion race of D. dipsaci), use of effective bio-fumigant cover crops and others.

## EFFECT OF SPACE-LIKE ENVIRONMENT ON THE BIOLOGY OF *CAENORHABDITIS ELEGANS*. Abdel-Rahman, Fawzia, N.M. Alaniz, S. Heydari, and B.A. Wilson. Department of Biology, Texas Southern University, Houston, TX 77004.

Different populations of the nematode *Caenorhabditis elegans* were exposed to simulated microgravity using the High Aspect Ratio Vessels (HARV) bioreactor which creates Low shear modeled microgravity (LSMM). C. elegans in liquid cultures (S medium seeded with E. coli OP50 as the food source), were exposed to simulated microgravity for different time intervals of 3, 6, 9, and 12 days, and 4, 8, 12, and 16 days. Control was kept in tissue culture vessels on a shaker. The bioreactor and the shaker were housed inside an incubator on 21 °C. After each exposure the total final population for each treatment and the control were determined and compared to the initial populations. All different developmental stages (eggs, L1, L2, L3, L4, and adults) were determined and counted in each treatment and the control. The survival and mortality were estimated. Several L3/L4 were selected from each treatment to study the brood size and the life span for each hermaphrodite. Individual L3/L4 was placed on NGM plate seeded with E. coli, once first egg was observed the adult was moved to a new and fresh plate of NGM seeded with E. coli to prevent overlapping of generations, and it was continued to be moved every two days until laying eggs was stopped, after that the adult was moved to a fresh plate and was observed daily until it died. All the eggs and hatched larvae were counted after each transfer to determine the brood size for each adult hermaphrodite. Eggs were extracted from gravid adult hermaphrodites (exposed and control) using alkaline bleach; eggs were placed on NGM plates seeded with E. coli, eggs were observed for development and hatching. Several adult hermaphrodites from each treatment and the control were processed to pure glycerin and mounted on glass slides to obtain measurements to determine their development. The molecular probe 4', 6-diamino-2-phenylindole dihydrochloride (DAPI) which stains the DNA and fluoresce under the fluorescence microscope was used to determine the effect of modeled microgravity on the number of the germ cells (germline) in the gonads of the adult hermaphrodites in both treated and control. The survival of C. elegans was reduced and the mortality was increased with the extended exposure to simulated microgravity. Eggs took longer time to develop and to hatch, and hatching rate was much lower than the control. The brood size was reduced for the hermaphrodites which were exposed to the low shear modeled microgravity, and it took longer time for the adults to start laying eggs, they deposited fewer eggs and the eggs were deposited for a shorter time (days) comparing to the control. Locomotion of the exposed animals was greatly reduced. Microscopic studies revealed that some of the morphometrics values were smaller in the simulated microgravity exposed C. elegans than the control.

INFLUENCE OF PLANT GROWTH PROMOTING RHIZOBACTERIA (PGPR) ON TOMATO PLANTS PERFOR-MANCE AND ROOT KNOT NEMATODE REPRODUCTION. Abdelmoneim, Tamer<sup>1,2</sup> and Samia Massoud<sup>2</sup>. <sup>1</sup>Biology Department, Faculty of Science, King AbdulAziz University, P.O. Box 15758, Jeddah 21454, Saudi Arabia. <sup>2</sup>Suez Canal University, Faculty of Agriculture, Department of Agricultural Botany, P.O. Box 41522, Ismailia, Egypt.

Numerous species of soil bacteria which flourish in the rhizosphere of plants or around plant tissues stimulate plant growth and reduce nematode population by antagonistic behavior. These bacteria are collectively known as PGPR (plant growth promoting rhizobacteria). The effect of six isolates of PGPR *Pseudomonas putida*, *P. florescens*, *Serratia marcescens*, *Bacillus amyloliquefaciens*, *B. subtilis* and *B. cereus*, were studied on tomato plant growth and root knot nematode reproduction after 45 days from nematode infection. The highest shoot dry weight (43.00g) was detected in the plants treated with *S. marcescens*; then *P. putida* (34.33g), *B. amyloliquefaciens* (31.66g), *P. florescens* (30.0g), *B. subtilis* (29.0g), *B. cereus* (27.0g) and nematode alone (untreated) 20g/plant. While the highest plant height was observed in plants treated with *S. marcescens*, *P. florescens*, *P. putida*, *B. amyloliquefaciens* and *P. putida* 52.66, 50.66, 48 and 48 cm respectively. No significant differences occurred between treatments but only significant differences compared with untreated plants. The highest number of fruit/plant was observed when plants treated with *S. marcescens* (10.66), then *B. amyloliquefaciens* (8.66), *P. putida* (8), *P. florescens* (8) and *B. cereus* (7.66). No significant differences between the last 4 treatments, but all were significant differences compared with untreated plants. The highest weight of plant yield (g) was observed with *S. marcescens* (319.6 g/plant) and the lowest weight of plant yield was observed in plants treated alone (untreated). On the other hand, the lowest numbers of J2/10g of soil (78), galls/root, (24.33) galls/root, egg masses/root (12.66) and egg/egg masses were observed on the plants treated with *S. marcescens*.

TYPE SPECIMEN USE IN NEMATODE TAXONOMY: AN EVALUATION OF RECENT LITERATURE WITH SUMMARY OF TYPE SPECIMENS DEPOSITED AT SMITHSONIAN NATIONAL MUSUEM OF NATURAL HISOTRY. **Abebe, Eyualem.** Department of Biology and Marine Environmental Science, Elizabeth City State University, Elizabeth City, NC 27909.

Despite recent advances & the wide use of DNA and molecular data in taxonomy, morphology remains to be a crucial component of species delimitation in nematode taxonomy. In light of morphology's critical importance to identification and better understanding of functional ecology of taxa, the importance of museum type specimens in determining the taxonomic identity of living organisms and documenting biodiversity cannot be overemphasized. Nevertheless accessibility of type specimens remains to be a limiting factor in their restricted use. To have a quantified overview of use of type specimens we assessed a portion of the nematode taxonomic literature of the past 13 years (1999-2011). Our results showed that of the 300 taxonomic papers we evaluated, only 2.5% of them reported comparative study of type material for decision on identity of target taxon. The remaining overwhelming majority (i.e. 97.5%) relied on comparisons with literature only. A detail discussion of problems reported in a sample of papers is discussed. Also, we provide a summary of the type material deposited at the Smithsonian National Museum of Natural History nematode collection. The NMNH nematode collection covers a truly global geographic range including specimens from the various oceans of the world bordering the continents of North America, South America, Asia, Africa, Europe and the Atlantic deep sea. It currently curates type specimens of 219 species widely spread in half of the 19 orders and a fifth of the 222 nematode families. The collection is impressively comprehensive in its taxonomic breadth at the family level in that nearly all (95%) of the commonly occurring 41 marine nematode families are well represented. We propose E-typing of nematode collections as one way of addressing the accessibility problem.

BUGWORMS.COM. Adams, J.<sup>1</sup>, W. Baughman<sup>1</sup>, G. Bird<sup>1</sup>, B. Eshchanov<sup>1</sup>, B. Glover<sup>1</sup>, C. Hahn-Townsend<sup>1</sup>, M. Kates<sup>1</sup>, J. Keven<sup>1</sup>, S. Mambetova<sup>2</sup>, P. Nelson<sup>1</sup>, J. Riddle<sup>1</sup>, P. Samota<sup>1</sup>. <sup>1</sup>Dept. of Entomology and <sup>2</sup>Dept. Crop & Soil Sciences, Michigan State University, East Lansing, MI 48824.

During spring semester of 2012, the Department of Entomology at Michigan State University (MSU) offered a one credit graduate-level seminar course entitled, Arthropod Nematology. This was the first time the topic of arthropod nematodes was ever taught at MSU as a stand-alone unit of academic instruction. Seven graduate students and three undergraduates enrolled. The students were instructed to develop an e-course on arthropod nematodes. The first part of the semester was spent researching distance learning technologies and educational philosophies. The e-domain bugworms.com was acquired for the student's product. Bugworms.com is designed for use as a self-tutorial e-course on arthropod nematodes, an e-reference for arthropod nematology courses, or as a miscellaneous hit during a www search. Bugworms.com consists of an introduction and ten units of instruction. Each unit contains a set of educational objectives, study questions, references and a highlight box, in addition to the subject matter related to each topic. The unit titles include: 1) Coleoptera: A Biodiversity Introduction, 2) Isoptera: An Insect-Nematode Relationship, 3) The Thunder Worm, 4) Social Behavior Transformation, 5) Mosquito Murderers, 6) Behavior Modification Induction, 7) Flies, Worms and Blindness, 8) A Fungal Channel that Works, 9) Red Rings and Orange Needles and 10) Of Germs and Worms. Some of highlights include: the types of nematode-insect relationships with special reference to fungal (*Deladenus siricidicola*) and bacterial ecosystem channels; nematode-induced

changes in insect, behavior (*Sphaerularia bombi*); insect morphology, color and social caste system alterations; synchrony of host-parasite relationships with weather events (*Mermis nigrescens*); near ubiquity of tri-trophic interactions; potential for use of as biological control agents for pest management and disease vector control programs. Research since the 2003 sequencing of the *Photorhabdus luminescens* genome (bacterial symbiont of *Heterorhabditis bacteriophora*) provides land mark insights into the nature of an extremely diverse array of toxicants. With the exception of the entomopathogenic species, arthropod nematodes have been significantly under-researched during the past few decades. While molecular evidence indicates that nematodes were in existence one billion years ago, the vast majority of the evolutionary history of nematodes comes from the more recent fossil record of arthropod nematodes. The students of ENT 812 sincerely hope that bugworms.com will in some small way catalyze an increased interest in the amazing domain of arthropod nematodes.

VARIABILITY AMONG TOBACCO CYST NEMATODE SUBSPECIES AND CONSEQUENCES ON THEIR SUS-TAINABLE MANAGEMENT WITH HOST RESISTANCES. Alenda, Charline<sup>1,2</sup>, D. Fouville<sup>2</sup>, A. Gallot<sup>2</sup>, N. Mariette<sup>2</sup> and E. Grenier<sup>2</sup>. <sup>1</sup>ANITTA, Domaine de la Tour, 24100 Bergerac, France, and <sup>2</sup>INRA, UMR1349 IGEPP, F-35653 LE RHEU, France.

The tobacco cyst nematode (TCN) is a highly specialized and sedentary pathogen composed of at least 3 subspecies: Globodera tabacum tabacum, Globodera tabacum solanacearum and, Globodera tabacum virginiae. These subspecies display no clear morphological polymorphism but are characterized by different developmental abilities in *Nicotiana* plants. Resistance to tobacco cyst nematode has become a promising alternative to the restricted use of nematicides, but the subspecies described in *Globodera tabacum* suggest that different efficiencies through time and/or through space will occur depending on the resistance used. Sustainable management of TCN thus requires accurate identification of the subspecies distribution and further investigations on the mechanisms set up by each resistance. In this study, we first investigated the intraspecific molecular variability of *Globodera tabacum* nuclear genes. One housekeeping and three parasitism genes were sequenced in a panel of 12 TCN populations. Analysis of the full-length sequences of the parasitism genes (one coding for an expansin and two for CLE peptides) revealed a high level of intraspecies variability (from 5,2% to 7,2%). Interestingly, the nucleotidic diversity in coding regions appeared not to be similarly distributed among subspecies which allow, as expected, the development of specific PCR-RFLP markers for 2 of the 3 subspecies. In a second step, we compared nematode and plant responses during interactions between one representative population of each subspecies and 3 Nicotiana resistances used in breeding programs. The effect of the root exudates on hatching was assessed and strong differences of response were observed for root exudates of resistant genotypes compared to sensitive genotypes. In addition, roots of these resistant genotypes were challenged with larvae of the different subspecies to specify the resistance spectrum and compare the plant mechanisms involved after larvae penetration (i.e. sex ratio imbalanced towards males vs. development restricted to J2/J3). The results, coupled with the subspecies markers, would help tobacco producers to apply the appropriate resistance in a given place and design sustainable crop rotations with resistances involving different mechanisms.

BACTERIA ANTAGONISTS TO PLANT NEMATODES IN EGYPT. Ali, Ali, H. Nematology Div., Faculty of Agriculture, Cairo University, Egypt.

Several bacteria and actinomycetes were isolated from *Meloidogyne* spp., egg masses and *Heterodera zeae* cysts. Screening the isolates for nematoxic effects resulted in four species with antagonisms to plant parasitic nematodes. The isolates were *Clostridium butyricum*, *Desulfovibrio desulfuricans*, *Pseudomonas fluorescence*, and *Streptomyces anulatus*. They were frequently associated with both egg masses and cysts. Liquid cultures (LCs) of the four isolates at concentration as low as 0.2% inhibited hatching of *M. javanica* eggs. The culture at a concentration of 0.6% were highly toxic to juveniles of *Meloidogyne javanica* and *Rotylenchulus reniformis*. All the tested microorganisms were responsible for considerable reduction in root and soil populations, maturation and reproduction of both nematodes.

HOST SUITABILITY OF PEPPER GENOTYPES TO *MELOIDOGYNE INCOGNITA*. Safdar A. Anwar<sup>1</sup>, M. M. Mahdi<sup>2</sup>, and M. V. McKenry<sup>3</sup>. <sup>1</sup>Institute of Agricultural Sciences, University of the Punjab, Quaid-e-Azam Campus, Lahore, 54590, Pakistan. <sup>2</sup>Pest Warning and Quality Control, Agricultural Department, Chinoit, Jhang.<sup>3</sup>Department of Nematology, University of California, Riverside, CA 92521, USA.

*Meloidogyne incognita* is a major pest of vegetable crops in Pakistan and has been reported to parasitize most members of the plant kingdom. The purpose of this study was to increase our understanding of host suitability among various pepper (*Capsicum annuum*) genotypes common to Pakistan. There were fourteen chili peppers genotypes including Gola Peshawari, C-19, 15-2010, 11-2010, C-68, Sanam, 27-2010, Tata Puri, C-302, C-73, C-72, 28-2010, 5-2010 and 18-2010 and five cultivars of bell pepper including Pahuja seeds (F1), Yolo Wonder, Orible, CDK-1000 and Capastreniou,. Three-week old plants of pepper genotype were transplanted into 13-cm dia. clay pots containing formalin sterilized sandy loam soil (70% sand, 22% silt, and 8% clay). Seven days after transplanting, a nematode suspension of 5000 eggs was poured into four holes about 3-cm deep around the base of each plant. The holes were then filled with soil and a little water was added to the pots. The check plants were inoculated with water only. There were three replicates of each genotype. The plants were irrigated

after every two weeks with Hoagland's solution. Host and nematode responses were evaluated after 60 days. Eggs were extracted from each root system and counted to determine final population density for each plant. Host suitability was categorized as good, fair, poor and non-host on the basis of reproduction factor [Pf = final population / Pi = initial population], root gall severity [on 0 to 9 scale], number of J2 per g of root, total number of eggs per root system, and root galling indices [on 0 to 5 scale]. The roots of all genotypes produced galls of variable number and size in response to nematode infection. Pepper genotype C-19 was ranked as a poor host with Pf/ Pi <1 and root gall severity of zero. Eight genotypes were categorized as a good host including Sanam, Gola Peshawari, 15-2010, 11-2010, C-68, Tata Puri, C-302 and 28-2010. Five other genotypes including 27-2010, C-73, C-72, 5-2010 and 18-2010 were ranked as fair hosts (Pf/Pi > 1). Two of five bell pepper genotypes were listed as poor hosts (Pf/Pi > 0) and included Orible and Pahuja seeds (F1). Genotypes Yolo Wonder and Capastreniou were fair hosts (Pf/Pi > 1) and genotype CDK-1000 a good host with Pf/Pi > 5.0.

ESTABLISHMENT RATE OF TOLERANT BERMUDAGRASS GERMPLASM IN A FIELD INFESTED WITH *BELONOLAIMUS LONGICAUDATUS*. Aryal, Sudarshan K.<sup>1</sup>, W.T. Crow<sup>1</sup>, R. McSorley<sup>1</sup>, R.M. Giblin-Davis<sup>1</sup>, and K.E. Kenworthy<sup>2</sup>. <sup>1</sup>Department of Entomology and Nematology, University of Florida, Gainesville, FL 32611; and <sup>2</sup>Department of Agronomy, University of Florida, Gainesville, FL 32611.

Reliance on a chemical option as the only management strategy for pest management is never an ideal situation. Use of bermudagrass (Cynodon dactylon) cultivars resistant or tolerant to Belonolaimus longicaudatus is essential for sustainable turf management in sandy coastal soils of the southeastern United States. Identification and development of tolerant bermudagrass cultivars and development of new IPM programs for plant-parasitic nematodes on golf courses in Florida are possible. Our objective was to determine whether B. longicaudatus-tolerant bermudagrass genotypes identified in previous greenhouse screening and field trials would establish more quickly in an infested field than the standard bermudagrass cultivar 'Tifway'. A multi-year field experiment including five bermudagrass genotypes and four different nematicide regimes in a split-plot design with five replications was initiated in fall, 2011. The five bermudagrass genotypes evaluated were Tifway, two commercial cultivars ('TifSport' and 'Celebration') that were identified as tolerant to B. longicaudatus, and BA 132 and PI 291590, which are experimental germplasm identified as tolerant to B. longicaudatus. Turf establishment was measured by digital image analysis to determine the percent of each plot covered by green turf every two weeks during the bermudagrass growing season. Turf establishment data from the first year are presented herein. Analysis of variance and comparison of means using Duncan's multiple range tests indicated differences in establishment rate among genotypes. In April, 2012, turf establishment was greatest for TifSport and Tifway (23% and 22% establishment, respectively) and lowest for BA 132 and Celebration (17% establishment each). Work is ongoing to follow these trends and to determine long-term performance of these germplasm entries in this site under heavy nematode pressure.

MOLECULAR DIAGNOSTICS OF ROOT-KNOT NEMATODES ON *PITTOSPORUM TOBIRA* IN FLORIDA. **Baidoo**, **Richard<sup>1</sup>**, **T.M. Mengistu<sup>1</sup>**, **J.A. Brito<sup>2</sup>**, **and W.T. Crow<sup>1</sup>**. <sup>1</sup>Entomology and Nematology Department, University of Florida, PO Box 110620, Natural Area, Dr., Gainesville, FL 32611; and <sup>2</sup>Florida Division of Plant Industry, 1119 SW 34<sup>th</sup> St., Gainesville, FL 32608.

Several ornamental crops are known to be susceptible to root-knot nematodes (*Meloidogyne* spp). A survey is on-going to identify *Meloidogyne* spp occurring on five cut foliage and ornamental crops, central to Florida's green industry. From each plant root collected, a single and five root-knot nematode females were used for genomic DNA extraction. PCR was carried out using a primer set to amplify the region between the cytochrome oxidase subunit II gene and the large subunit of the 16S ribosomal RNA gene in the Mitochondrial DNA. A single fragment of about 1.7kb was produced for both single females and the five females collected from roots of *Pittosporum tobira*. Restriction Fragment Length Polymorphism (RFLP) of the 1.7 kb fragment using Hinf I restriction enzyme produced a three-banded pattern characteristic of *Meloidogyne incognita*. The survey is on-going and additional *Meloidogyne* spp are likely to be detected.

## AMBUSH FORAGING ENTOMOPATHOGENIC NEMATODES EMPLOY 'SPRINTING EMIGRANTS' FOR LONG DISTANCE DISPERSAL IN THE ABSENCE OF HOSTS. **Bal, Harit K., Robin A.J. Taylor, and P.S. Grewal.** Department of Entomology, OARDC, The Ohio State University, Wooster, OH 44691.

Ambush foragers must employ some long-distance dispersal strategy to maximize reproductive success in the absence of hosts. To test this hypothesis we compared lateral dispersal of the ambusher, *Steinernema carpocapsae* and cruiser, *Heterorhabditis bacteriophora* from infected host cadavers in sterilized silt loam soil in microcosms (0.05 m<sup>2</sup>-1.5 m<sup>2</sup>) with or without vegetation in the absence of hosts. Nematode dispersal was estimated by taking soil cores (5 by 2 cm dia) from the microcosms at different intervals (6-240 h) and distances (3.8-61 cm) from the infected host cadavers and baiting with *Galleria mellonella* larvae. The numbers of baited larvae killed and the numbers of infective juveniles penetrated in dead baits were counted and analyzed by  $\chi^2$  contingency tables. Results revealed that vegetation enhanced dispersal of both species but more so in *H. bacteriophora*. Average population displacement was similar (~6 cm/day) for both species but the

pattern of dispersal differed spatio-temporally. Nearly all *H. bacteriophora* dispersed away (3.8-61 cm) from the cadaver, but majority (66%) of *S. carpocapsae* remained within 8 cm of the cadaver. Interestingly, a small proportion (0.2%) of *S. carpocapsae* dispersed much faster and farther than *H. bacteriophora*. This use of apparently 'sprinting emigrants' may represent an adaptive dispersal strategy by the otherwise ambush forager *S. carpocapsae* in the absence of hosts. The difference in the degree of movement (both active and passive) of the two EPN species in the field, determined in two different habitats, potato and adjoining grassy border over distance and time, compared with the results obtained from laboratory experiments will be presented. Such quantitative information on nematode movement generated from laboratory and field experiments would help in filling the gap in the knowledge on spatial and temporal dynamics of these beneficial nematodes and establishing sustainable pest management programs and conservation approaches for using these biological control agents.

INVENTORY OF THE SOIL NEMATODE FAUNA AT WHITE SANDS NATIONAL MONUMENT, NEW MEXICO. **Beacham, Jacqueline<sup>1</sup>, S. Thomas<sup>1</sup>, and E. Bernard<sup>2</sup>.** <sup>1</sup>Department of Entomology, Plant Pathology, and Weed Science, New Mexico State University, Box 30003, MSC 3BE, Las Cruces, NM, 88003; and <sup>2</sup>Department of Entomology and Plant Pathology, University of Tennessee, 2431 Joe Johnson Drive, Room 205, Knoxville, TN, 37996-4500.

The rare gypsum landscapes at White Sands National Monument (WHSA), located in southern New Mexico, represent unique chemical and physical habitats for both soil microorganism and plant communities. Activity and diversity of soil organisms are expected to play key roles in the sustainability of the ecosystem. This site is characterized by seasonal, ephemeral precipitation, and thus variable available nutrient inflow, followed by localized or generalized drought. These events combined with low water-holding capacity of arid soils and rapid depletion of available nutrients induces a succession of short, wet, likely copiotrophic periods followed by extended dry and oligotrophic stages. Superficial aquifers induce similarly variable gradients across depth. The purpose of this inventory was to assess how soil nematode populations differ across the gradient of ecological sites at WHSA, and to discover if these environments lead to unique soil nematode diversity or speciation. To answer these questions, six ecological sites were inventoried across a gradient of gypsiferous dune development at WHSA including: mesquite coppice dunes of the quartzose sand sheet at the edge of gypsum sand dunes; the barren area associated with the dry lakebed of Lake Lucero where the gypsum sands originate; an interdune cottonwood site; marginal Atriplex-grassland site; intermittent playa site; and an extremely active barchan dune area. Rhizosphere soils from three plants of each of the two dominant plant species and three, non-rhizosphere, depth-stratified soil samples (0-10 cm; 10-30 cm) were collected at each site. Samples were collected during the fall of 2011 at the end of the monsoon season, when soil microorganism abundances are at their peak, permitting evaluation of maximum biotic potential. Soil classification, fertility and gravimetric moisture content were also assessed and recorded for each site. Plant-parasitic nematodes and Aphelenchidae were initially identified to genus; all other nematodes were quantified by trophic category with microscopy. Extracted nematodes were preserved and mounted for morphological species identification with representative specimens photographed and catalogued for inventory purposes. No living nematodes were collected from either of the lakebed sites or the non-rhizosphere cottonwood soils. Fungivores were dominant in the top 10 cm of non-rhizosphere soil at both the quartzose sand-sheet site and the active dune site. However, plant-rhizosphere soils at these two sites differed, with herbivores and bacterivores present in high numbers at the quartzose site while the fungivore population remained predominant at the active dune sight. Surprisingly few nematodes were recovered from the cottonwood-rhizosphere soil despite the prevalence of roots. Herbivores were collected in extremely high numbers from the Atriplex-grassland site in comparison with the abundance of the other trophic groups. This nematode fauna inventory is the first report for the largest gypsum dune field in North America.

CALORESPIROMETRIC ANALYSIS FOR OPTIMIZING SEED COATINGS. Becker, J. Ole<sup>1</sup>, and D. Burger<sup>2</sup>. <sup>1</sup>Department of Nematology, University of California, Riverside, CA 92521; and <sup>2</sup>Department of Plant Sciences, University of California, Davis, CA 95616.

Seed coatings with compounds that improve crop performance in the presence of plant pathogens or pests are important tools to prevent or mitigate crop diseases and damage. In addition to one or more active ingredients, seed coatings include components such as stickers, dyes, metal pigments, nutrients, filling and flowing material, and others. Multi-component products often require time-consuming and labor-intensive optimization studies as the ingredients may affect individual pesticidal efficacy and directly or indirectly compromise seed germination, seedling development and response to pathogens and pests. We evaluated calorespirometric analysis of germinating seeds as a potential tool for optimizing multi-component seed coatings. Industry-provided pesticide-coated vegetable seeds were used as test samples. Imbibed seeds were placed into a differential scanning calorimeter operated in isothermal mode  $(25^{\circ}C)$  to measure rates of heat production. The calorimeter had four chambers; three were used for simultaneous measurements of rate of heat production while the remaining chamber served as a reference. Rates of  $CO_2$  evolution by the respiring seeds were determined by trapping the gas in 0.4 M NaOH. Heat produced by the exothermic reaction between  $CO_2$  and NaOH, forming carbonate, was measured to estimate respiration rates. Time requirement for an analysis run was less than 20 min. The analysis was faster and more sensitive than germination tests and proved to be useful to eliminate coatings with negative effects on the metabolism of germinating seeds.

*BRACHYPODIUM DISTACHYON*: A MODEL PLANT TO STUDY ROOT-KNOT NEMATODE-POOIDEAE INTER-ACTIONS. **Becker<sup>1</sup>**, **J. Ole, H. Witte<sup>1</sup>**, **J. Smith Becker<sup>1</sup>**, **G. W. Douhan<sup>2</sup>**, **J. P. Vogel<sup>3</sup>**, and **A. Ploeg<sup>1</sup>**. <sup>1</sup>Department of Nematology, University of California, Riverside, CA 92521; <sup>2</sup>Department of Plant Pathology and Microbiology, University of California, Riverside, CA 92521; and <sup>3</sup>USDA-ARS Western Regional Research Center, Genomics and Gene Discovery Unit, Albany, CA 94710.

Brachypodium distachyon (purple false brome) is an annual grass of Mediterranean origin but worldwide distribution. It belongs to the grass subfamily Pooideae that includes cool season cereal crops, forage and turf grasses. With favorable characteristics such as small size, fast cycling, inbreeding, compact genome, and diverse diploid accessions, it has been championed as a model for cereal crops and biofuel grasses. We are investigating its research utility for studies with root-knot nematode species. Seeds of various inbred lines were imbibed in water and decontaminated in 10% bleach. After one week of moist incubation at 4°C, germinated seed was planted in sandy soil or soilless growth pouches. The growth media were infested with second-stage juveniles of either *Meloidogyne incognita* race 1 or *M. graminis* and incubated at  $23\pm2^{\circ}$ C in ambient light. In the growth pouches, slight root swellings were observed after 15-17 days and eventually the enlarging females broke through the root surface. Although egg production on the different B. distachyon lines were quite variable, most were better hosts for M. graminis than M. incognita with an average of about 300 eggs/egg mass and approximately 5 times higher egg population density than *M. incognita* based on root length. The first eggs of both species were observed in the growth pouches after 27 days. In many growth pouches fungal growth occurred that developed on plant material and produced abundant dark and hairy globose perithecia. The fungus was identified by ITS rDNA sequence analysis as Chaetomium globosum, a common endophyte and seedborne fungus associated with many plant species. Although no obvious effects on root-knot nematodes were observed, its potential influence on pathogen-host interactions requires further investigations.

FIELD DAMAGE CAUSED BY *PRATYLENCHUL ALLENI* ON SOYBEAN IN QUEBEC, CANADA. Bélair, G., and B. Mimee. Agriculture and Agri-Food Canada, St-Jean-sur-Richelieu, Quebec, Canada J3B 3E6.

In 2011, irregular yellowish/chlorotic soybean patches with reduced growth were observed for the first time in a 10-ha soybean field grown on a light texture soil in St. Anicet, Quebec. Previously in corn, this field was sown with soybean PRO 2715R, a Roundup Ready® cultivar. Soil analysis revealed a uniform pH of 6.0 on a gravely sandy soil. On October 7, 2011, plants were harvested and, soil and root samples were collected from 10 randomly selected damaged patches. From each patch, 3 samples were systematically recovered, one from the center of the patch, and 2 samples on the ridge of the patch, exhibiting normal growth. Five additional random samples were collected from a symptomless zone as control. A 1x1m quadrat was used as the sampling unit area. The numbers of nematodes in roots were estimated by placing fresh roots in a mist chamber for a 14-day extraction period. Nematode specimens were stored in tap water at 4°C before identification. Based on morphological characters, twenty-six specimens were examined (14 females, 11 males, one juvenile) and were all identified as Pratylenchus alleni Ferris, 1961. Yield of each plot was also recorded after drying the plants at 67°C for 4 days. Our results showed a yield reduction of 46% in symptomatic plots (2.16 t/ha) compared with controls (4.05 t/ha) and 41% decrease when compared to the edge of the patch (3.65 t/ha). Root samples showed an average of 1119±259 P. alleni/g dry root in damaged zones while 335±111 and 524±104 nematodes/g dry roots were recovered from control and ridge respectively. Pratylenchus alleni was found in all soil and root samples. In the United States, the pathogenicity of P. alleni in soybean has been well established. This is the first report of field damage caused by a root-lesion nematode in Quebec and is also the first detection of P. alleni in Canada.

AN UNDESCRIBED SPECIES OF ANGUINIDAE PARASITIZING THE MOSS *HYPNUM CUPRESSIFORME*. Bernard<sup>1</sup>, Ernest C., J. Hentschel<sup>2</sup>, and P.G. Davison<sup>3</sup>. <sup>1</sup>Entomology and Plant Pathology Dept., University of Tennessee, 2431 Joe Johnson Drive, Room 205, Knoxville, TN 37996-4500; <sup>2</sup>Friedrich Schiller University, Dept. of Systematic Botany, Herbarium Haussknecht (JE), Fürstengraben 1, 07740 Jena, Thüringen, Germany; and <sup>3</sup>Box 5232, Dept. of Biology, University of North Alabama, Florence, AL 35632.

Nematode-induced galls on mosses have been reported many times in the literature, but in only a few instances have the nematodes been studied and described. The moss *Hypnum cupressiforme* var. *cupressiforme* was collected in November 2011 at Bad Salzungen, Thuringia, dried with heat, and prepared for the herbarium. Soon thereafter terminal swellings were noted and dissection demonstrated that the swellings contained females, males, and second-stage juveniles. Several galls were dissected in water. One female, several males and about 30% of J2 were revived one hour after immersion. This species was placed tentatively in *Anguina* as the female was slightly swollen and heat-relaxed in a near circular pattern (length 1.2 mm), but these are not definitive *Anguina* characters and the taxon also could fit in *Subanguina*. Internally the female was badly degraded and the reproductive tract could not be studied; however, the metacorpus in all stages appeared to be much more substantial than that illustrated for other moss-galling Anguinidae. Males were similar to those of *Subanguina brenani* (=*Anguina brenani*), described from Oxford, England, on the moss *Pottia bryoides*, but were longer (1.1-1.2 mm in the undescribed species, 0.6-0.7 mm in *S. brenani*), and the stylet and spicule were longer (13 µm and 32-34 µm vs. 10 µm and 25-26 µm). Male and J2 tail tips

were rounded in the undescribed species but sharply pointed in *S. brenani*. Juveniles were 728-889  $\mu$ m long and their stylet lengths were 10-12  $\mu$ m; *S. brenani* juveniles were not described. The undescribed species resembles *Subanguina askenasyi*, also described from *H. cupressiforme*, but doubt exists about the limits of this species. In the original description Bütschli stated that female and male lengths were 1.7 mm and 1.4 mm, respectively, but Steiner gave lengths of 0.98-1.2 mm and 0.92-1.2 mm, with a stylet length in both sexes of 10  $\mu$ m. Despite the similarity in measurements it is unlikely that Steiner's specimens are the same taxon as the undescribed species, since stylet length is different. In addition, the tail tips of Steiner's specimens are sharply pointed, whereas in the undescribed species they are rounded. Separation of *Anguina* from *Subanguina* is dependent on the structure of the female reproductive system. Additional specimens, especially of live, gravid females, are needed to study morphology of the gonad for definitive placement in *Anguina* or *Subanguina*.

NON-DESTRUCTIVE X-RAY IMAGING OF ROOT SYSTEMS INFECTED WITH ENDOPARASITIC NEMATODES. **Bernard, Ernest C.<sup>1</sup>, D.W. McDonald<sup>2</sup>, R. Michaels<sup>2</sup>, and B.H. Ownley<sup>1</sup>.** <sup>1</sup>Entomology and Plant Pathology Dept., University of Tennessee, 2431 Joe Johnson Drive, Room 205, Knoxville, TN 37996-4500; and <sup>2</sup>Phenotype Screening Corp., Suite 10, 4028 Papermill Rd., Knoxville, TN 37909.

Observation of nematode-induced root disease is hampered by the opacity of soil and other growing media and by the need for sufficient replication to allow statistically meaningful but destructive sampling. Observation of symptom development on roots in an X-ray-transparent medium was explored through non-destructive two-dimensional X-ray imaging of cotton and sunflower seedlings. Seedlings were grown in germination pouches and root systems were inoculated with freshly hatched M. incognita juveniles after initial lateral root emergence (3-4 days after germination). After gall initiation, infected seedlings were taken to the growth and X-ray facility at Phenotype Screening and transplanted into an X-ray-transparent substrate consisting of 0.5-1-mm expanded polystyrene beads. Plants were watered frequently with Hoagland's solution and maintained under a 14-hour photoperiod. Root systems were periodically and non-destructively X-rayed to observe root architecture, root growth and gall development. Gall enlargement was successfully observed over a 27-day period. Nematodes developed to the female stage on sunflower and produced egg masses by 30 days after inoculation. Nematodes did not appear to be harmed by repeated X-rays, and eggs obtained from X-rayed females hatched and developed normally on tomato. In another study, sunflower roots infected with Rotylenchulus reniformis in the greenhouse were carefully washed and the root systems were X-rayed. Egg masses were visible in the X-ray images as distinct hemispheres on the root with a darker ring around the egg mass base compared to the rest of the egg mass. This approach has great potential for studying nematode development and resistance, and the influence of root architecture on nematode parasitism. In addition, stereo X-ray imaging of cotton roots heavily laden with R. reniformis egg masses indicated a high potential for rapid counting of egg masses or attached endoparasitic nematodes.

### EVOLUTION OF VIRULENCE IN AN ENTOMOPATHOGENIC NEMATODE SYMBIONT. Blackburn, Dana, and B.J. Adams. Dept. of Biology 401 WIDB, Brigham Young University, Provo, UT 84602.

Entomopathogenic nematodes (EPNs) are important biological control agents consisting of two genera, Steinernema and Heterorhabditis, which kill their insect hosts with the help of their bacterial symbionts, Xenorhabdus and Photorhabdus, respectively. *Photorhabdus* is a genus of Gram-negative bacteria belonging to the Enterobacteriaceae family. In addition to forming a mutualistic relationship with the Heterorhabditidae family of nematodes, these bacteria are primarily responsible for insect mortality during the nematode infection. Photorhabdus virulence is dependent on various toxins and other virulence factors common to this family of bacteria such as proteases and type III secretion systems. There are three described species of *Photorhabdus*; *luminescens* and *temperata*, which are strictly entomopathogens, and *asymbiotica*, which has been isolated from wound infections in humans. Phylogenetic relationships were investigated using parsimony and maximum likelihood analyses with 62 taxa and three genes, 16S rRNA, gyrB, and glnA. Species formed strong monophyletic groups; however, subspecies placement was not as resolved. To investigate how virulence has evolved in this genus, bacterial cells were injected into Galleria mellonella larvae, and the  $LT_{50}$  was calculated for each strain. These values were mapped onto the phylogeny using ancestral reconstruction methods. The results show that high virulence may have evolved multiple times in this genus and it might be strain dependent. Additionally, virulence-associated genes were investigated for selective pressures to provide further understanding of the evolution of virulence in this organism. Understanding how virulence has evolved in this bacterium will aid in unraveling the mechanisms of the Heterorhabditis-Photorhabdus complex, which may aid in the selection of nematode-bacterium complexes for biological control.

## HOST REACTION OF BRAZILIAN COMMON BEAN UPRIGHT CULTIVARS TO *MELOIDOGYNE INCOGNITA* AND *PRATYLENCHUS BRACHYURUS*. **Bonfim Junior, Mauro and M.M. Inomoto.** Plant Pathology and Nematology Dept., University of São Paulo, Piracicaba, SP, Brazil 13418-900.

Common bean (*Phaseolus vulgaris*) is often attacked by *Meloidogyne incognita* and *Pratylenchus brachyurus* in areas where it is cultivated. In Brazil, the breeding program of common bean has been directed for the establishment of upright cultivars due to the increasing demand, in the last years, for plants that are easily harvested mechanically. This work dealt with the assessment of some Brazilian common bean upright cultivars, which could eventually be rated as sources of genes

#### 454 Journal of Nematology, Volume 44, No. 4, December 2012

for nematode resistance, in relation to M. incognita and P. brachyurus. In a trial referred to as Experiment 1, twenty cultivars (BRS Campeiro, FT Soberano, IPR Chopim, IPR 139, BRS Estilo, BRS Radiante, IAPAR 81, IPR Tuiuiú, BRS Xamego, IPR Uirapuru, IPR Corujinha, IPR Juriti, BRS Agreste, IPR Graúna, IPR Tiziu, BRS Esplendor, IPR Gralha, IPR Tangará, IAC Diplomata and BRS Valente) were inoculated (Pi) with 300 M. incognita (eggs+J2)/plant and the susceptible controls were cotton 'Fibermax 966' and maize 'DKB 330'. In the experiment 2, nine cultivars (BRS Valente, BRS Campeiro, IPR Tuiuiú, IPR 139, FT Soberano, IPR Chopim, BRS Estilo, BRS Radiante and IAPAR 81) were inoculated with 300 P. brachyurus (eggs + motile forms)/plant and the susceptible controls were soybean 'Pintado' and common bean 'Pérola'. In both trials, the experimental design was completely randomized with six replications and they extended for 60 days until final evaluation. Data of final population (Pf), nematodes/g of roots (Nem/g) and nematode reproduction factor (RF = Pf/Pi) were recorded. In the experiment 1, all the cultivars were rated as susceptible to *M. incognita* ( $RF \ge 1$ ). Among the common bean cultivars, IPR Corujinha showed the lowest RF (5.86) and Nem/g (158) values. The Rf values of the cotton 'Fibermax 966' and the maize 'DKB 330' were 8.05 and 2.70, respectively. In the experiment 2, all the cultivars were susceptible to P. brachyurus. The cultivars BRS Valente and BRS Campeiro showed the lesser RF (5.26 and 5.38, respectively) and Nem/g (142 and 138, respectively) values. The RF values determined for the soybean 'Pintado' and common bean 'Pérola' were of 4.25 and 14.88, respectively. From these results it was inferred that no source of resistance to the nematodes tested was detected among all the common bean upright cultivars assessed.

#### AN EFFECTOR D26 MAY BE INVOLVED IN THE SUPPRESSION OF PLANT DEFENSE THROUGH THE IN-TERACTION WITH FTR-C. **Borong, Lin, K. Zhuo, and J.L. Liao.** Laboratory of Plant Nematology, South China Agricultural University, Guangzhou, China 510642.

*Meloidogyne* is one group of important plant pathogenic nematodes, causing considerable losses of many crops in the world. Some valuable studies have been conducted to investigate the molecular mechanism involving in nematode infection. Reactive oxygen species (ROS), especially hydrogen peroxide ( $H_2O_2$ ), has been proven to be related to the expression of defense-related genes by the nematode infection in the nematode-plant interaction. A gene *D26* encoding for a 125-aa protein with a predicted molecular mass of 14.3 KDa was isolated from *M. javanica*. The effector has been shown to be a secreted protein from esophageal gland. Over expression of *D26* rendered *Arabidopsis* susceptible to *M. javanica*. The yeast two hybrid screening and BIFC were conducted to indicate that the protein interacts with FTR-c in *Arabidopsis thaliana*. Constitutive expression of *D26* in *Arabidopsis thaliana* reduced the  $H_2O_2$  content. The effector protein added to *Arabidopsis* crude extracts can promote  $H_2O_2$  degradation. A correlation was observed between D26-mediated  $H_2O_2$  degradation and the abundance of PTR-c transcripts on *Arabidopsis* crude extracts. Expression of *D26* in *Arabidopsis* significantly reduced the abundance of PR-1 to PR-5. It can be concluded that the effector D26 may be involved in the suppression to plant defense through the interaction with FTR-c, but further research is needed in the future.

GENETIC STRUCTURE OF GOLDEN NEMATODE POPULATIONS FROM QUEBEC, CANADA, WITH THE USE OF NEW MICROSATELLITE MARKERS. Boucher, Annie Christine<sup>1</sup>, B. Mimee<sup>1</sup>, J. Montarry<sup>2</sup>, G. Bélair<sup>1</sup>, P. Moffett<sup>3</sup>, and E. Grenier<sup>2</sup>. <sup>1</sup>Agriculture and Agri-Food Canada, St-Jean-sur-Richelieu, Quebec, Canada J3B 3E6; <sup>2</sup> Institut national de la recherche agronomique, Le Rheu, France 35653; and <sup>3</sup> Université de Sherbrooke, Sherbrooke, Quebec, Canada J1K 2R1.

The golden nematode (*Globodera rostochiensis*), originally from South America, has been introduced in many parts of the world, including North America. In 2006, it was found for the first time in the province of Quebec, Canada in the locality of St. Amable near Montreal. So far, very few studies have examined the population genetics of this pest. Consequently, a lack of knowledge exists on the structure and evolution of global populations of the golden nematode. In our study, twelve new microsatellite markers were developed to answer these questions. These markers were used to genotype fourteen populations originating from different regions, including two from Quebec. Within populations, the greatest genetic diversity has consistently been observed in the populations from Bolivia, the region of origin of the golden nematode. The two Quebec populations were very similar to each other but, surprisingly, were significantly different from other North American populations including those from New York and British Columbia. Indeed, these populations appear to be genetically closer to European populations in general, and to a Scottish population in particular. Based on our results, the golden nematode has been introduced in North America at least twice, from at least two other regions.

*MELOIDIGYNE* FATTY ACID AND RETINOL BINDING PROTEIN (MjFAR) IS REQUIRED FOR DEFENSE SINGNALLING MANIPULAITON DURING PARASITISM. **Brown Horowitz, S.**<sup>1\*</sup>, **Iberkleid, I.**<sup>1,2</sup>, **Ozalvo, R**<sup>1</sup>. **Vieira, P**<sup>3</sup>., **de Almeida Engler, J**<sup>3</sup>. **and Spiegel, Y**<sup>1</sup>. <sup>1</sup>Department of Entomology and Nematology and Chemistry units; ARO, the Volcani Center, Bet Dagan, 50250, Israel; <sup>2</sup>Department of Plant Pathology and Microbiology, the Faculty of Agriculture Food & Environment, the Hebrew University of Jerusalem, Rehovot, 76100, Israel; and <sup>3</sup>UMR Institut Sophia Agrobiotech INRA/CNRS/UNS, 400 route des Chappes, 06903 Sophia Antipolis, France.

Root-knot nematodes, *Meloidogyne* spp., are highly destructive pathogens that exhibit a sophisticated interaction with plants governed by continuous signal exchange among them. Herein, we exploit the lipid signaling dialogue between plants

and nematodes and their ability to manipulate and perceive the fatty acid oxidation products, oxylipins. In plants, oxylipins are synthesized by lipoxygenases (LOXs) and dioxygenase (DOX) via enzymatic oxidation of linoleic or linolenic acids. Our findings indicate that gene expression of *Solanum lycopersicon* (tomato) belonging to the 9-LOX and  $\alpha$ -DOX group are differentially regulated upon the root-knot nematode *M. javanica*, infection. Moreover, altered fatty acid desaturation by suppressing the tomato fatty acid desaturase 3 gene (*FAD3*) results in decreased susceptibility to *M. javanica*, as indicated by lower counts of adult females compared to *FAD3* overexpressing line and wild-type. These results prompted our hypothesis that distinct branches of the LOX/DOX pathway can either serve for host defense or be manipulated by nematodes to suppress defense, presumably via secretion of some pathogen-derived effectors. Herein, one group of nematode effectors, the fatty acid and retinol binding (FAR) proteins, which interferes with the plant's LOX-mediated defense signaling by binding LOX/DOX substrates and products, was studied. Thus far, our findings indicate that the *M. javanica* FAR encoding gene is up regulated throughout the parasitic stages. The localization of the MjFAR during parasitism by immunocytochemistry further provides compelling evidence for its involvement in plant defense manipulation by nematodes. Thus, we present here an in-depth characterization of the role of FAR in eliciting local suppression of LOX-defense pathways to promote successful infection.

POTENTIAL OF *NEOACTINOLAIMUS* AS A BIOLOGICAL CONTROL AGENT OF ROOT-KNOT AND RENIFORM NEMATODES. Cabos, Roxana<sup>1</sup>, K-H. Wang<sup>2</sup>, and I. Wang<sup>2</sup>. <sup>1</sup>USDA, ARS, U.S. Pacific Basin Agricultural Research Center, 64 Nowelo Street, Hilo, HI 96720; and <sup>2</sup>Department of Plant and Environmental Protection Sciences, University of Hawaii at Manoa, 3050 Maile Way, Honolulu, HI 96822.

The predatory nematode Neoactinolaimus spp. (family Actinolaimidae) was examined as a potential biological control agent against root-knot (Meloidogyne spp.) and reniform (Rotylenchulus reniformis) nematodes in laboratory conditions. Neoactinolaimus possesses a large odontostylet to puncture the cuticle of its nematode prey and feed on their contents. Neoactinolaimus was selected for this experiment due to the high abundance recovered from the rhizosphere of Hawaiian native sedge, 'Ahu'awa Cyperus javanicus. In vitro cultures were established on quarter strength corn meal agar (CMA/4) containing carrot discs and bacterial feeding nematodes dominated by Rhabditidae as prey. The reproductive rate of Neoactinolaimus in this CMA/4 culture varied from 0 to 16 nematodes/month. An in vitro assay was conducted using soil nematodes extracted from a field previously planted in cantaloupe (Cucumis melo) and highly infested with root-knot (Meloidogyne incognita and M. javanica) and reniform (Rotylenchus reniformis) nematodes. Soil was extracted using an elutriator and the centrifugal flotation method. All nematodes extracted were identified to genus level and counted before and 6 days after the introduction of 16 Neoactinolaimus per beaker. Five replicated beakers were used. The Neoactinolaimus were then picked and frozen for molecular gut analysis using multiplex PCR primers targeting the ITS region of Meloidogyne spp. and R. reniformis. The experiment was repeated once. Assuming that all the nematodes that disappeared 6 days after inoculation was due to the feeding of *Neoactinolaimus* as no other nematode predators were present in the beakers except omnivorous nematodes, *Neoactinolaimus* suppressed 60% and 48% of the population of *Meloidogyne* spp. and *R. reniformis*, respectively in Trial I; and suppressed 34% and 61%, respectively in Trial II. Suppression of bacterivores, fungivores, herbivores and omnivores by Neoactinolaimus were 76, 21, 87, and 81%, respectively in Trial I; and were 54, 51, 48, and 78%, respectively in Trial II. Confirmation of the feeding on Meloidogyne spp. and R. reniformis by Neoactinolaimus using multiplex PCR analysis is in progress. Further research is needed on culture media that can support more consistent reproduction of *Neoactinolaimus*, and an in vivo assay on suppression of *Meloidogyne* spp. and *R. reniformis* by *Neoactinolaimus*.

MANIPULATING SOIL FOOD WEBS IN A FLORIDA ORGANIC CITRUS ORCHARD TO ENHANCE BIOCON-TROL BY ENTOMOPATHOGENIC NEMATODES. **Campos-Herrera, Raquel<sup>1,2</sup>, F. E. El-Borai<sup>1,3</sup>, L. W. Duncan<sup>1</sup>**. <sup>1</sup>Citrus Research and Education Center, University of Florida, 700 Experiment Station Rd, Lake Alfred FL 33850; <sup>2</sup> Instituto de Ciencias Agrarias, CSIC, Serrano 115 dpdo Madrid, 28006, Spain; and <sup>3</sup>Plant Protection Department, Faculty of Agriculture, Zagazig University, Egypt.

An emerging organic citrus industry in Florida could benefit greatly from effective, nonconventional methods to mitigate losses from pests and diseases. We are investigating the effects of OMRI (Organic Materials Review Institute) approved cultural practices on soil food webs in order to develop methods to conserve and enhance biological control of insect pests by indigenous entomopathogenic nematodes. An experiment was established in a commercial, organic citrus orchard on Florida's central ridge. The treatments consisted of three amendments: i) a mulch of commercially pelleted chicken manure, ii) a commercial formulation of *Paecilomyces lilacinus* applied twice, iii) sulfur applied twice to reduce soil pH, and iv) an unamended control. The experimental design was complete randomized block with 10 replications, each comprising three adjacent trees. Soil samples were taken pre-application and 1, 3 and 6 months post-treatment initiation (T0-T6). Response variables included adult root weevils (*D. abbreviatus*) emerging from soil, soil pH and moisture, citrus fibrous roots dry weight, free-living (FLN) and plant-parasitic nematodes (PPN), and measurement by real-time PCR of 6 entomopathogenic nematode (EPN) species, 5 species of nematophagous fungi (NF), 2 *Paenibacillus* bacterial species that are phoretic on EPNs and *Acrobeloides*-group nematodes that can compete with EPNs. The only EPNs detected were *Steinernema diaprepesi*,

*Heterorhabditis indica* and *H. zealandica*. Seasonal increase in numbers of *H. zealandica* (P < 0.001) and *Acrobeloides*-group (P < 0.001) were not affected by treatments. All treatments decreased *H. indica* and *Hirsutella rhossiliensis* compared to controls at T1 (P = 0.040, and 0.019, respectively). Numbers of *Paenibacillus* sp. were directly related to both those of *S. diaprepesi* (P = 0.026) and *Acrobeloides*-group nematodes (P < 0.001). Similarly, *Paenibacillus nematophilus* was directly related to *H. indica* (P = 0.026) and *Acrobeloides*-group nematodes (P < 0.001). Similarly, *Paenibacillus nematophilus* was directly related to *H. indica* (P = 0.001). At T3 FLNs were more numerous in plots mulched with manure (P = 0.014). The NF *Paecilomyces lilacinus* increased in plots where it was augmented (T1 P = 0.053; T3 P < 0.001; T6 P = 0.021), reaching a maximum level at T3 that was 17.5-fold greater than that in controls. At the same time (T3), two indigenous NF, *Arthrobotrys dactyloides* and *Monacrosporium gephyropagum*, tended to increase (P = 0.051 and P = 0.078, respectively) in *P. lilacinus*-amended plots, as did the DNA recovered from nematode samples and citrus fibrous root weights (P = 0.003 and P = 0.022). However, elevated levels of these NF had no effect on any of the nematode trophic guilds (EPNs, phytoparasites, or free living nematodes) at any time during the first year of this ongoing experiment.

#### OVERVIEW OF NATIONAL AND INTERNATIONAL NEMATODE COLLECTIONS. Carta, Lynn K. USDA ARS Nematology Laboratory, Beltsville, MD, 20705.

Nematode collections are invaluable resources for taxonomy, evolution, speciation, ecology, biodiversity, conservation, climate change, invasive species, emerging diseases and trade. Voucher specimens are also crucial for scientific reproducibility. The housing and curation of many collections have been poorly supported for some time. The role of collections is changing with addition of new kinds of virtual, sequence, and georeference data. The locations of some have changed over the years, such as the recent transfer of Calvin Massey's insect nematode collection to the University of Nebraska. An update of collections resources is needed to better use and support them. Therefore, a summary of information on preserved and live collections are surveyed from the *Caenorhabditis* Genetics Center to an assortment of international labs associated with the Worm Systematics Research Network. The condition and needs of these various collections are discussed. Information is also provided on initiatives to integrate collection databases and digitize collections such as the NSF-funded iDigBio project of interest to formal and informal nematode collection managers.

### TAXONOMY AND PHYLOGENY OF SOME *PANAGROLAIMUS* NEMATODES ASSOCIATED WITH AERIAL PLANT PARTS. Carta, Lynn K., and A.M. Skantar. USDA ARS Nematology Laboratory, Beltsville, MD, 20705.

Most species of *Panagrolaimus* are considered saprophytes, often found in soil or rotting plant material. However their considerable anhydrobiotic ability has allowed at least a few of them to exist and even thrive within living, aerial plant parts such as leaves, seeds and stems. These include *P. rigidus* associated with coconut leaf rot in India, *Panagrolaimus* cf. *rigidus* and *Helminthosporium* associated with melting-out of turfgrass in North Dakota and Minnesota, and an unidentified species from India that reduced viability of pearl millet seeds. Another seed-associated population is a new species of tiny *Panagrolaimus* that was discovered in large numbers associated with seeds of the lipstick tree *Bixa orellana* originating in Ghana and provided through the APHIS port of Milwaukee, WI in October, 2011. After a day in distilled water, seeds yielded hundreds of tiny nematodes, with a small minority actively moving that were placed in bacterial culture and morphologically characterized. A *Panagrolaimus* species originally described in association with a butterfly from Russia, *P. artyukhovskii*, was recently discovered in association with *Hydrangea* sp. stems originating in Canada provided through the APHIS port of Detroit, MI, in February, 2011. The same species was found living as an endophyte within stems of cheatgrass and *Fusarium* from Colorado. Another *Panagrolaimus* population was found within stems of alfalfa from Utah. These plant-associated *Panagrolaimus* populations were processed for molecular sequencing with ribosomal DNA and Hsp90 genes and placed in context with other GenBank accessions of *Panagrolaimus* species to examine their phylogenetic distribution.

## EFFECT OF *BACILLUS FIRMUS* GB-126 AGAINST *ROTYLENCHULUS RENIFORMIS*, *MELOIDOGYNE INCOGNITA*, AND *HETERODERA GLYCINES* UNDER *IN VITRO* AND GREENHOUSE CONDITIONS. Castillo, J.D., D. Schrimsher, and K. Lawrence. Department of Entomology & Plant Pathology, 209 Life Science, Auburn University, AL36849.

The bacterium *Bacillus firmus* GB-126 has been reported reducing numbers of plant-parasitic nematodes, however its mechanism of activity remains unclear. The objective of this work was to evaluate under *in vitro* the effect of *B. firmus* cells and cell-free extracts have on egg hatching and second stage juveniles of *R. reniformis*, *M. incognita*, and *H. glycines*. Treatments for cell free extract were: 1) water control, 2) media control, 3) cell-free extract 100%, and 4) cell-free extract 50%. Treatment for bacterium cells were: 1) water control, 2) media control, 3) *B. firmus* 1x10<sup>7</sup> cfu/ml. Each trial consists of five replications per treatment and was repeated twice. Bacterium cell-free extracts at a concentration of 50% and 100% inhibits *R. reniformis* egg hatching at 48 and 72 hours after inoculation, while second stage juveniles appeared paralyzed after 1h of inoculation ( $P \le 0.001$ ). Furthermore, cell-free extracts at a concentration 50% and 100%, and bacterium cells at a concentration of 1x10<sup>7</sup> cfu/ml significantly inhibited egg hatching of *M. incognita* and *H. glycines* at 96 hours after inoculation through 9 days after inoculation, respectively. The results of this study indicate that bacterium cells and cell-free

extracts can have a direct effect on *R. reniformis*, *M. incognita*, and *H. glycines* reducing the egg hatching and paralyzing and possible killing second stage juveniles.

*HSP90* GENE EXPRESSION OF *MELOIDOGYNE INCOGNITA* AND *HETERODERA GLYCINES* UNDER TEMPER-ATURE STRESS. Chen, Lijie, F. Zhu, X.F. Zhu, F. Wang, Q.Zou, D.Wang, Y.Y. Wang, and Y.X. Duan. Nematology Institute of Northern China, College of Plant Protection, Shenyang Agricultural University, Shenyang 110866, China.

The heat shock protein, *Hsp90*, is highly conserved among different nematodes. To study gene expression induced by temperature stress (cold and heat) in Meloidogyne incognita and Heterodera glycines, qRT-PCR experiments using genespecific primers were carried out. Females and second-stage juveniles (J2) of M. incognita were exposed at 4° and 35° during different time intervals in order to explore the impact of adverse temperature on Mi-hsp90 gene expression. At 35°C the levels of J2 Mi-hsp90 transcription were 27.9-fold higher than those of the J2 control after 2h of heat shock treatment; subsequently, expression dropped, but still remained 22.6-fold higher than that of the control after 24h. Exposure of females to heat stress (35°) increased the relative levels of Mi-hsp90 transcript by 5.5-fold to 6.1-fold after 1h to 24h. Exposure of J2 to cold (4°) increased the relative levels of Mi-hsp90 transcript by 9.2-fold and 11.0-fold after 1h and 24h. However, cold-stress only slightly changed the relative abundance of Mi-hsp90 mRNA in females. Expression levels of Mi-hsp90 at 35° in females and J2 exceeded those of *Mi-hsp90* at 4°. This finding clearly demonstrates that *Mi-hsp90* is necessary as a defense mechanism against high temperature. Cysts of H. glycines, as the survival stage in soil, are often exposed to environmental stresses. Consequently, we tested  $H_g$ hsp90 gene expression at different levels of temperature (-20°, 4°, 25° and 35°) on the cyst. Exposure of cysts to heat shock (35°) slightly increased the level of mRNA of Hg-hsp90 transcript by 2.3-fold after 24h. In cold exposure (4°) cysts showed upregulation of the level of Hg-hsp90 such that after 24h exposure it was 2.0-fold higher than that of the cyst control. In subzero exposure (-20°) cysts showed a 7.0-fold increase of Hg-hsp90 after 1h of cold exposure; subsequently, the Hg-hsp90 mRNA level dropped but still remained 4.5-fold higher than that of the control cysts. These results indicate that cysts respond rapidly to cold and thus suggest that *Hg-hsp90* plays important roles on survivability in cold environments.

## IMPACT OF TILLAGE AND SOURCE OF RESISTANCE ON DYNAMICS OF THE SOYBEAN CYST NEMATODE POPULATION AND ITS VIRULENCE PHENOTYPE. **Chen, Senyu.** University of Minnesota Southern Research and Outreach Center, 35838 120th St, Waseca, MN 56093.

The soybean cyst nematode (SCN), *Heterodera glycines*, is the most damaging pathogen of soybean. Use of resistant cultivars is the most common practice in managing the nematode. Currently, most (approximately 95%) commercial SCNresistant soybean cultivars in the North Central United States were developed from a single source of resistance, Plant Introduction (PI) 88788. The resistance of a small portion of cultivars was from Peking and PI 437654. The selection pressure of SCN-resistance on SCN populations may differ with different sources of resistance. A field plot experiment was initiated in 2003 to study the effect of tillage and source of resistance on SCN population density and virulence phenotype. The initial SCN population in the field was HG Type 0-, which is avirulent to the three sources of resistance. The main aim of the project was to determine how different sequences of the three cultivars Latham EX547 RR N, 91M90, and AR5084, carrying SCNresistance from PI 88788, Peking, and PI 437654, respectively, influence the changes of the SCN population from the initial avirulent HG Type to a virulent type on cultivars carrying the three sources of resistance. Tillage had little effect on SCN virulence phenotype and population density. As expected, SCN population densities differed following different sequences of the soybean cultivars, and susceptible soybean resulted in greater egg population density than resistant cultivars. Among the three SCN-resistant cultivars, the PI 88788-derived cultivar supported the greatest SCN egg population density, the Peking-derived cultivar was intermediate, and the PI 437654-derived cultivar supported the smallest egg population density. Based on the HG Type analysis of the populations collected in 2007, 2008, 2009, and 2010, SCN populations (HG Type 2-) selected by the PI 88788-derived cultivar overcame the resistance of PI 88788 but not the other two resistance sources, and the Peking-derived cultivar selected SCN populations (HG Type 1-) that overcame the resistance in Peking. In contrast, the PI 437654-derived cultivar selected SCN populations (HG Type 1.2-) that overcame both PI 88788 and Peking sources of resistance. There was no increase of Female Index (FI) on PI 437654 in any cultivar sequence before 2010. However, FI on PI 437654 of the SCN populations from the monoculture of the PI 437654-derived cultivar in conventional tillage plots increased slightly (FI = 3) in 2010. No clear pattern of effect of rotations of the three sources of resistance on virulence phenotype was observed during the 8 years of study. This study will continue to determine longer treatment effects.

A COMPARISON BETWEEN URBAN AND AGRICULTURAL SOILS USING SOIL NEMATODE COMMUNITY AND OTHER KEY SOIL PARAMETERS. **Cheng, Zhiqiang<sup>1</sup>, R. Islam<sup>2</sup>, S.S. Briar<sup>3</sup>, and P.S. Grewal<sup>1</sup>.** <sup>1</sup>Department of Entomology, The Ohio State University, OARDC, Wooster, OH 44691; <sup>2</sup>The Ohio State University South Centers, Piketon, OH, 45661; and <sup>3</sup>Department of Soil Science, University of Manitoba, Winnipeg, Manitoba, Canada, R3T2N2.

Soils in urban ecosystems are often considered highly degraded due to intensive human activities. In this study, we compared soils from urban vacant lots, turfgrass lawns, and community gardens to soils in rural agroecosystems in Northeast Ohio using data on a diversity of soil chemical and biological parameters published in our previous studies. Additionally, an

improved soil quality index was developed based on diverse soil chemical and biological parameters from both urban and agricultural soils. The results indicated that NH<sub>4</sub>-N, NO<sub>3</sub>-N, microbial biomass, soil organic matter, nematode abundance (total, and all trophic groups), and nematode food web enrichment index were significantly higher, but nematode plantparasitic index was lower in urban soils than in agricultural soils. Principal component analysis (PCA) revealed a similar pattern that urban vacant lots had higher NH<sub>4</sub>-N and soil organic matter than urban turfgrass lawns and agroecosystems, and urban community gardens had higher NH<sub>4</sub>-N and soil organic matter than agroecosystems. Therefore, in contrast to the conventional belief, urban soils in Northeast Ohio are as good or even better than agricultural soils in many soil quality parameters. These properties coupled with their high nitrogen content suggest that urban soils have high potential to support urban agriculture. In addition, soil parameters contributing most to soil quality were identified by PCA, and an improved soil quality index was then deduced using these key identified biotic and abiotic parameters, which were NH<sub>4</sub>-N, SOM, total nematode abundance, free-living, bacteria-feeding, fungal-feeding, omnivorous and predatory nematode abundance. This improved soil quality index revealed similar difference between urban and agricultural soils as the combination of all measured soil parameters did, and thus has potential to serve as a comprehensive and effective indicator of overall soil quality.

EFFECTS OF LONG-TERM TILLAGE AND ROTATION ON THE RELATIONSHIPS BETWEEN *HETERODERA GLYCINES* AND SOIL NEMATODE COMMUNITY. **Cheng, Zhiqiang<sup>3</sup>, S. Mennan<sup>1,2</sup>, P.S. Grewal<sup>3</sup>, and H. Melakeberhan<sup>1</sup>.** <sup>1</sup>Agricultural Nematology Laboratory, Department of Horticulture, Michigan State University, East Lansing, MI 48824; <sup>2</sup>TUBITAK Visiting Scholar from Ondokuz Mayis University, 55139 Samsun, Turkey; and <sup>3</sup>Department of Entomology, The Ohio State University, OARDC, Wooster, OH 44691.

The soybean cyst nematode (SCN, *Heterodera glycines* Ichinohe) is a serious pest of soybeans worldwide. Although there is substantial knowledge on SCN biology, we know little about the potential biological mechanisms by which the agronomic practices affect SCN in the soil. It is important to quantify the biological mechanisms by which SCN thrives under biological and physiochemical changes driven by agricultural practices in order to develop sustainable ecosystem management strategies. In this study, a factorial experiment consisting of tillage, rotation and SCN infestation was initiated in July 2001 in a location where SCN has never been reported and soybeans were not grown before. There were two tillage treatments (chisel plow, and no-till), five rotation treatments (monocropping corn (C), monocropping SCN- resistant (R) soybean, monocropping SCN-susceptible (S) soybean, RCRC rotation, and SCSC rotation), and two nematode treatments (SCN-infested, and No SCN). This paper focuses on the latest data collected in 2008 and 2009. The results indicate that SCN population had positive correlations with total nematode abundance, total non-SCN nematode abundance, free-living nematode abundance, and bacteria-feeding nematode abundance in both years. This suggests that conditions favoring free-living nematodes can also favor SCN. Further analysis between nematode populations and food web and various management strategies (tillage, SCN infestation, rotations) suggests that in addition to direct impacts, tillage and SCN infestation may have indirect impacts on increasing SCN population through decreasing free-living nematodes.

### IMPROVING SOYBEAN RESISTANCE TO THE SOYBEAN CYST NEMATODE- A COMPREHENSIVE SYSTEM APPROACH. Cianzio, S.R. Raymond F. Baker Center for Plant Breeding, Iowa State University, Ames, IA.

Among the pathogenic species of nematodes threatening soybean (*Glycine max*) production, the soybean cyst nematode (*Heterodera glycines;* SCN) is the most damaging. The release of cultivars resistant to SCN is therefore of utmost importance, since genetic resistance is the most efficient mode to control the pathogen and protect soybean yield. Classical breeding techniques have successfully delivered resistant cultivars and continue to do it. However, molecular tools applied to both biological entities, soybean and nematodes, have opened up possibilities for increasing breeding efficiency. This presentation will address the current use of technologies. It will also address how the advent of new molecular information and technologies may further contribute to improve breeding efficiency in soybean for resistance to the soybean cyst nematode.

### NEW SPECIES OF *CALOOSIA*, *CRICONEMA*, *HEMICYCLIOPHORA* AND *MESOCRICONEMA* (NEMATODA: CRICONEMATOIDEA). Cordero, Marco and R.T. Robbins. Plant Pathology Department, 2601 N. Young Ave. Cralley-Warren Research Lab. University of Arkansas, Fayetteville, AR. 72704.

New species of Criconematoidea found in Arkansas and North Carolina between 2008-2011 are reported. Nematodes were killed and fixed in hot 3% formaldehyde, and subsequently infiltrated with glycerin using Seinhorst's modified slow method. Morphological characterization was performed by obtaining the nematode's morphometrics represented by measurements and ratios using light microscopy (Nikon Optophot-2) and a Nikon Drawing Tube. *Caloosia* n. sp. was found associated with pines and *Paspalum* sp. is characterized by having a long body (1081.3-1431.3 µm), long stylet (105.6-123.8 µm), flattened body annuli (R=338-357), interruptions in body annuli laterally that demarcate a single lateral field without lines and a simple rounded vulva slit (V=75.8-77.8), without modified vulva lips. *Criconema* n. sp. 1 was found associated with hackberry, *Paspalum* sp., maple and oatgrass. The species has a conspicuous head offset from the body with two annuli, short rounded

tail with a thin cuticular sheath and subterminal anus. *Criconema* n. sp. 2 was found associated with *Paspalum* sp. Specimens have two cephalic annuli about the same width, first annulus directed posteriorly, separated by a narrow neck annulus and a short conoid tail with a unilobed non-folded annulus. *Hemicycliophora* n. sp. was found associated with turfgrass in North Carolina and is characterized by having a lateral field demarked by two faint lines with transverse anastomoses and/or breaks of the striae, long vulva lips and vulval sleeve, an elongated, non offset, conical tail, with distinct annulations ending in a rounded tip. *Mesocriconema* n. sp. was found associated with *Paspalum* spp. and is characterized by having small flattened submedian lobes, lower than labial disc or at the same level, vagina straight, very well developed rounded spermatheca without sperm, no more than one anastomoses, L= 379-512  $\mu$ m, V=88.7-92.9, stylet length = 48.7-60.9  $\mu$ m, R=107-119, annuli with crenate margins at tail portion and a simple anterior vulva lip. ITS-1 amplicons were obtained and submitted to GenBank. The genus *Caloosia* has been reported in India, Bangladesh, Sri Lanka, South Africa, Fiji, and Hawaii islands. This is the first report of the genus in North America.

### PREPARE YOURSELF FOR A JOB YOU LOVE. Crow, William T. Entomology and Nematology Department, University of Florida, PO Box 110620, Natural Area, Dr., Gainesville, FL 32611.

Most nematologists did not plan on going into Nematology when they started their college career. Instead, they were exposed and infected somewhere along the way, finding it interesting, challenging, or for some mysterious reason loving it! Within Nematology there are many career paths and specializations that utilize a variety of skills and talents. Fortunately, each graduate student has unique skills, talents, and interests; they are not all the same. Graduate school offers a great opportunity to identify strengths and weaknesses, likes and dislikes, to determine what kind of career you want and to prepare for it. This is a time to try new things. There might be fascinating areas within Nematology that await your discovery. On the other side, it is best not to waste time spending ten years in college preparing to do something that does not interest or excite you. Instead, prepare for a job you love. Find out what makes you feel most alive; is it sequencing, farming, lecturing, modeling? Would you rather study a nematodes structure, biochemistry, or ecological niches? Or, maybe you just like to kill them! Do you prefer calling the shots, following instructions, or doing your own thing? Usually you excel at the things you like, so identify career paths that you are passionate about and prepare yourself for those jobs.

### EFFICACY OF MCW-2 AS A NEMATICIDE FOR TURF. Crow, William T. Entomology and Nematology Department, University of Florida, PO Box 110620, Natural Area, Dr., Gainesville, FL 32611.

MCW-2 (fluensulfone) is a new nematicide with a novel mode-of-action produced by Makhteshim Agan. In 2011, two field trials at the University of Florida evaluated a 1.5% a.i. granular formulation of MCW-2 as a post-plant nematicide for turf sod production and golf course uses. The sod trial was on 'Empire' zoysia (*Zoysia japonicum*) infested with damaging numbers of *Hopolaimus galeatus*. Treatments were MCW-2 1.5 G at 4 kg a.i./ha applied as a single application or split into two applications of 2 kg a.i./ha each applied 4 wk apart, and untreated control. Efficacy was evaluated by measurement of *H. galeatus* population density, and sod strength (measure of sod harvestability). The golf course turf trial was conducted on 'Tifdwarf' bermudagrass (*Cynodon dactylon*  $\times$  *C. transvaalensis*) infested with damaging numbers of *Belonolaimus longicaudatus*. Treatments were MCW-2 1.5 G at 4 kg a.i./ha applied as a single application, split into two applications of 2 kg a.i./ha each applied 4 wk apart, or split into 4 applications of 1 kg a.i./ha each applied 4 wk apart, or split into 4 applications of 1 kg a.i./ha reduced the population density of *H. galeatus* in the soil and improved sod strength compared to the untreated control. In the golf course experiment all the MCW-2 treatments reduced the population density of *B. longicaudatus*, and improved turf percent green cover and root length compared to the untreated control. These results indicate that MCW-2 could become a useful nematode management tool for turfgrass production and maintenance.

#### IDENTIFICATION OF WIDELY VARYING LEVELS OF RESISTANCE TO *MELOIDOGYNE INCOGNITA* IN SWEET SORGHUM. **Davis, Richard F., and W.F. Anderson.** USDA ARS, P.O. Box 748, Tifton, GA 31793.

Sweet sorghum (*Sorghum bicolor*) is a potential bioenergy crop that could be incorporated into annual cropping systems in the southern US, where it would likely be rotated with cotton. The desirability of including sweet sorghum in a cotton cropping system will be influenced by sweet sorghum's host status for *Meloidogyne incognita*, which is the primary pathogen of cotton in the US, but almost no information is available on the host status of sweet sorghum for *M. incognita*. However, grain sorghum, which is also *S. bicolor*, is reported as both a poor host and as a good host to *M. incognita*, and some reports state that sorghum is a host without reporting the amount of reproduction. We hypothesized that a broad range of resistance and susceptibility could be found in a genetically diverse collection of sweet sorghum entries. A series of greenhouse tests were conducted to evaluate the reproduction of *M. incognita* on 82 sorghum entries obtained from several different sorghum collections. Entries were arranged by height into four groups, and 19 to 22 entries were evaluated in each of four tests. Corn (*Zea mays*) was used as a susceptible standard in each test. Tests were conducted in a randomized complete block design with three replications. In each replication, three plants of a single entry were planted into a 15-cm-diam. pot. Two to three weeks

after planting, 8000 eggs of *M. incognita* were added to each pot. Nematode eggs were extracted 8 weeks after inoculation, and the number of plants/pot, total root weight/pot, and total eggs/pot were recorded, and eggs/plant and eggs/gram root were calculated. Mean root weights of the sorghum varied from 18 to 282% of the weight of corn roots. Nematode reproduction on sorghum ranged from 0 to 379% of the eggs per plant found on corn, and from 0 to 216% of the eggs/g root found on corn. Eggs/plant recovered from 30 (37%) of the 82 sorghum entries tested, and eggs/g root recovered from 29 (35%) of the entries, were less than 10% of the levels recovered from corn. This preliminary screen confirms that there is a very wide range of resistance to *M. incognita* among sweet sorghum genotypes.

## IDENTIFICATION OF ROOT-KNOT NEMATODE RESISTANCE LOCI IN *GOSSYPIUM HIRSUTUM* USING SIMPLE SEQUENCE REPEAT MARKERS. **Del Rio, Sonia, and J.L. Starr.** Department of Plant Pathology and Microbiology, 2132 TAMU, College Station, TX 77843-2132.

Gossypium hirsutum, also known as Upland cotton, is one of the major crops grown in the United States and across the world. The cultivation of Upland cotton occurs in areas with warm temperatures; areas that double as ideal breeding grounds for the southern root-knot nematode (RKN), Meloidogyne incognita. Meloidogyne incognita has a wide host range and distribution, making it a difficult plant pathogen to control. In the U.S., RKN can cause yield losses ranging from 10-25%. Further, infection by RKN increases the likelihood of a RKN-Fusarium wilt complex. Host plant resistance has shown to be the most effective way to control RKN populations. Currently, RKN resistance used in most breeding programs is derived from a few related sources. A half-diallele genetic study has shown the G. hirsutum lines TX-1174, TX-2107, and TX-2076 to be highly resistant to *M. incognita* when compared to Clevewilt-6. However, when compared to Wild Mexico Jack Jones, TX-1174 and TX-2076 had high levels of resistance whereas TX-2107 only had moderate levels of resistance. Resistance in TX-1174 and TX-2076 appears to be governed by two dominantly inherited genes whereas TX-2107 had resistance governed by one dominant gene. The objective of this study is to identify and map root-knot nematode resistance loci in these unique G. hirsutum lines (TX-1174, TX-2107, and TX-2076) using simple sequence repeat (SSR) markers. The lines used will be: 1) inoculated with *M. incognita*, 2) phenotypically analyzed by measuring the nematode reproduction as eggs per gram of fresh root and host response using a root gall index, 3) genetically evaluated by using SSR markers to detect polymorphisms between the three RKN resistant TX lines and DP90 (susceptible line), and 4) analyzed using linkage and mapping software. Genotypic analysis of these lines that would further support the half-diallele study is currently underway. To date, 48 of the 150 SSR markers that have been tested have shown polymorphisms between the three RKN resistant TX lines and the susceptible DP90. A bulked segregant analysis approach is being used to test resistant and susceptible bulks of the  $F_2$ population. The rest of the F<sub>2</sub> population will be tested if the SSR markers continue to indicate polymorphisms between the bulks. Identification of SSR markers linked to RKN resistance will facilitate marker-assisted selection in breeding programs where the goal is to develop new cultivars that contain RKN resistance.

HOST-SEEKING, OLFACTION, FORAGING STRATEGIES, AND THE GENOMIC ARCHITECTURE OF PARA-SITISIM AMONG *STEINERNEMA* NEMATODES. **Dillman<sup>1</sup>**, **Adler**, **Ali Mortazavi<sup>2</sup>**, **Elissa Hallem<sup>3</sup>**, **and Paul W. Sternberg<sup>1</sup>**. <sup>1</sup>Howard Hughes Medical Institute, Division of Biology, California Institute of Technology, Pasadena, CA 91125; <sup>2</sup>Developmental and Cell Biology, Center for Complex Biological Systems, University of California, Irvine, Irvine, CA 92697; and <sup>3</sup>Department of Microbiology, Immunology, and Molecular Genetics, University of California, Los Angeles, Los Angeles, CA 90095.

Many parasitic nematodes actively seek out hosts to infect and thus complete their lifecycles. Olfaction is thought to play an important role in the host-seeking process, with parasites following a chemical trail toward host-associated odors. Steinernema is a diverse genus of entomopathogenic nematodes, with over 60 described species. The host-seeking behaviors of Steinernema include chemotaxis and jumping behavior and vary along a foraging strategy continuum between cruise and ambush strategies. Little is known about the odors that stimulate host-seeking behavior or how olfactory information is interpreted in these agriculturally important parasitic nematodes. We explored the host-seeking responses of several Steinernema species to CO<sub>2</sub> and volatile organic compounds produced by laboratory and ecologically relevant potential hosts. We show that all *Steinernema* species tested are attracted to  $CO_2$ , but display varying behavioral responses to different potential hosts, suggesting that they can differentiate between potential hosts based on odor alone. CO<sub>2</sub> seems to play a key role in the host-seeking process for steinernematids, though this varies for different parasite-host combinations and for different host-seeking behaviors. Further, we used gas chromatography-mass spectrometry and solid-phase microextractiongas chromatography-mass spectrometry to identify odorants emitted by seven potential hosts. We found that these odorants stimulate host-seeking behaviors in a species-specific manner, suggesting that parasitic nematodes have evolved highly specialized olfactory systems to enable their navigation through complex environments and make appropriate host selection decisions. To pursue these observations further, we sequenced the genomes and transcriptomes of five Steinernema species (S. carpocapsae, S. scapterisci, S. monticolum, S. feltiae, and S. glaseri). In an unbiased search through the vast genomic data, we found striking evidence of expansions in protease, protease inhibitor, and G-protein coupled receptor families. Our results indicate that Steinernema species have experienced significant evolution of these protein families, provocatively suggesting that the repertoire of olfactory receptors and armory of proteases underlie niche partitioning and the evolution of foraging strategy and host range among *Steinernema* nematodes.

EFFECT OF BROILER LITTER APPLICATION TO SOYBEAN CROP INFESTED WITH SOYBEAN CYST NEMATODE. **Donald, Patricia<sup>1</sup>, P. B. Allen<sup>2</sup>, D. D. Tyler<sup>3</sup>, K. R. Sistani<sup>4</sup>, H. Tewolde<sup>5</sup>, and E. R. Walker<sup>6</sup>. <sup>1</sup>USDA ARS Crop Genetics Research Unit, Jackson, TN 38301; <sup>2</sup>BESS, University of Tennessee, Knoxville; <sup>3</sup>BESS University of Tennessee, Jackson, TN 38301; <sup>4</sup>USDA ARS Animal Waste Management Unit, Bowling Green, KY 42104; <sup>5</sup>USDA ARS Genetics and Precision Agriculture Research Unit, Mississippi State, MS 39762; and <sup>6</sup>University of Tennessee, Dept of Plant Science, Martin, TN 38238.** 

Manipulation of the plant root zone to reduce the impact of plant parasitic nematodes has been a goal of researchers. Addition of animal manure has a long history of improved soil quality and reduced soil-borne diseases. The objective of this research was to measure the agronomic benefits of broiler litter application to a soybean crop under no-till and disk regimes and to assess the impact of litter application on soybean cyst nematode (*Heterodera glycines*) reproduction. Greenhouse studies indicated a reduction in the number of *H. glycines* cysts after one month when dried broiler litter was added to pots. In a small-plot field study, two levels of broiler litter (6.7 Mg ha<sup>-1</sup> and 13.4 Mg ha<sup>-1</sup>) were either surface applied or incorporated into plots under different tillage regimes (disk tillage and no-till). Controls were without broiler litter in the disk and no-tillage plots and were monitored to detect changes in soil fertility necessitating the application of commercial fertilizer. Application of broiler litter for soybean growth as measured by plant height was observed regardless of the rate. Significant grain yield differences were observed in 2008 (P= 0.0025). Spectral measurements among treatments were highly significant in 2008 (P=0.0001). Spectral measurements were highly correlated with plant height, grain yield, and *H. glycines* egg population density at harvest in 2008. Despite positive preliminary research results from greenhouse studies, we could not measure a significant effect of broiler litter application on *H. glycines* in field trials.

THE FIRST REPORT OF *CACTODERA EREMICA* BALDWIN & BELL, 1985 (NEMATODA: HETERODERIDAE) IN CHINA. **Duan, Yuxi, D. Wang, X.F. Zhu, and L.J. Chen.** Nematology Institute of Northern China, Shenyang Agricultural University, Shenyang, Liaoning, China, 110866.

During a survey of cyst nematodes in North China from July to August 2011, several cysts were isolated by sievingdecanting methods from soil in the rhizosphere of Ulmus pumila from two natural areas (Hunhe riverside and Beiling Garden) in Shenyang, Liaoning Province, China. Cysts, eggs, and the second-stage juveniles (J2) were observed and measured using light microscopy and scanning electron microscopy. The results showed that these two populations agree well with Cactodera eremica. Cysts (n = 10) were lemon-shaped, light to dark brown color with protruding neck and small vulval cone; external cyst wall pattern at mid-body consisting of nearly straight to wavy transverse lines frequently interrupted by short oblique or vertical lines, circumfenestrate, abullate, vulva denticles absent, anus situated at the base of vulval cone, body length (including the neck) 481.6-766.1 (average 637.7)  $\mu$ m, body width 304.9-484.2 (425.4)  $\mu$ m, ratio of body length and width 1.3-1.6 (1.5), circumfenestral diameter 19.9-26.3 (23.5) µm and vulva to anus distance 24.4-32.4 (28.9)  $\mu$ m. J2 (n = 15) body cylindrical, elongate, tapering at posterior end, body length 425.3- 510.8 (469.0)  $\mu$ m, labial region slightly offset, bearing four or five annuli, with dorso-ventrally elongated labial disc and six labial sectors including four large submedian and two small distinct lateral lips, stylet length 23.5-26.0 (24.6) µm; knobs rounded to slightly projecting anteriorly; the anterior surface of stylet knobs slightly concave, the lateral field with four incisures; outer two ridges sometimes aerolated, tail with blunt end, 38.6-46.8 (43.3) µm long; hyaline tail terminal 16.8-21.9 (19.9 µm) long. Eggs (n=24) 99.2-126.9 (110.3) µm long, 38.4-61.6 µm (47.9) µm wide, exterior shell with small punctations forming a distinct pattern, visible under light microscopy and SEM. Molecular characterization of C. eremica is performed. The ITS products of ribosomal DNA were digested with eight restriction enzymes (AluI, AvaI, Bsh1236I, BsuRI, CfoI, MvaI, PstI and RsaI) to obtain RFLP profiles. This is the first report of C. eremica in Asia and China.

## SCANNING ELECTRON MICROSCOPY OF THE FEEDING SITE OF *MELOIDOGYNE KIKUYENSIS* ON SUGAR-CANE. Eisenback, J.D.<sup>1</sup>, and D. J. Dodge<sup>2</sup>. <sup>1</sup>Department of Plant Pathology, Virginia Tech, Blacksburg, VA 24061; and <sup>2</sup>Department of Biology, Bryan College, Dayton, TN 37321.

Galls on sugarcane caused by the kikuyu grass root-knot nematode, *Meloidogyne kikyensis* de Grisse, 1965, appear similar to nodules produced by nitrogen-fixing bacteria on leguminous plants. The galls are usually rounded and smooth or they may be covered with numerous fine root hairs. Scanning electron microscopy of dissected galls revealed a complex and unique feeding socket that is formed at a 90° angle to the vascular cylinder of the root to which the gall is attached. The gall is made up of two major components: the feeding socket that is composed of several giant cells that are ramified with vascular tissue and a larger gall formed from parenchyma cells that surrounds the feeding socket and the individual second-stage juvenile, adult female, or developing male. Usually galls caused by the majority of the root-knot nematodes are parallel with the vascular cylinder and do not form a specialized feeding socket. The structure of this feeding site gives credibility to the observation that some root-knot nematodes secrete proteins (NemF) proteins that are similar to proteins (NodF) secreted by

*Rhizobia* spp. and other nodulating bacteria. Whereas *M. kikuyensis* has been considered to be a primitive species within the genus because of its simple morphology and low chromosome number, the structure of its feeding site is very complex.

IMPACT OF TWO SEED TREATMENTS ON CORN YIELD AND POPULATION OF CORN PARASITIC NEMATODES IN ON-FARM TRIALS. **Faghihi, Jamal<sup>1</sup>, B. Bower<sup>2</sup>, R.L. Nielsen<sup>3</sup>, G.J. Bossaer<sup>4</sup> and V.R. Ferris<sup>1</sup>.** <sup>1</sup>Department of Entomology, Purdue University, West Lafayette, IN 47907; <sup>2</sup>Ceres Solutions, 1104 S 700 W, Lafayette, IN 47909; <sup>3</sup>Department of Agronomy, Purdue University, West Lafayette, IN 47907; and <sup>4</sup>White County Cooperative Extension Service, 12 N 25 E, Reynolds, IN 47980.

The high price of corn and the introduction of seed treatments have created new opportunities to study corn parasitic nematodes. Introduction of seed treatments to manage corn parasitic nematodes has created increased demand for universities to provide independent data and to verify the effectiveness of these products. In 2011, we established on-farm field plots in southern and northern Indiana to determine the effectiveness of two common seed treatment products (Avicta<sup>®</sup> and VoTivo<sup>®</sup>) and Counter<sup>®</sup> insecticide/nematicide for management of corn parasitic nematodes and their impact on corn yield. These plots were established based on previous history of these fields and the types of corn parasitic nematodes involved. We have monitored various species of corn parasitic nematodes throughout the growing season on these sites for several years. These plots were between 100 to 1100 feet long and were planted and harvested using grower equipment. At all locations, we had more than usual rainfall early in the season, which might have influenced the effectiveness of the various treatments and the stress caused by nematodes on corn. Our studies in 2011 on the corn seed treatments showed inconsistent results in managing corn parasitic nematodes were found in other plots where seed treatments were used. Similar effects on the populations of corn parasitic nematodes were found in other plots. At this site, the corn yield was significantly higher in the VoTivo<sup>®</sup> plots. No effects on corn yield or corn parasitic nematodes were found in the other northern plot.

*DE NOVO* TRANSCRIPTOME ASSEMBLY OF THE FOLIAR NEMATODE *APHELENCHOIDES FRAGARIAE*. **Fu, Zhen<sup>1</sup>, C.E. Wells<sup>1</sup>, G. Collier<sup>2</sup> and P. Agudelo<sup>1</sup>.** <sup>1</sup>School of Agricultural, Forest, and Environmental Sciences, 114 Long Hall, Clemson University, Clemson, SC 29634; and <sup>2</sup>Cyberinfrastructure Technology Integration, 321 Fluor Daniel Building, Clemson University, Clemson, SC 29634.

The foliar nematode *Aphelenchoides fragariae* (Aphelenchida: Aphelenchidae) is an endo- and ectoparasite that feeds on aerial parts of over 250 plant species from 47 families. It is a common and economically-damaging pest of nursery-grown crops including foliage and flowering plants. Management of foliar nematodes is challenging because of its facultative parasitism (i.e. having the ability to feed on both plants and fungi) and its ability to survive in low moisture conditions. We used Illumina HiSeq 2000 platform and the Trinity *de novo* assembler (trinitymaseq.sourceforge.net/) to produce a transcriptome of *A. fragariae* from four conditions (fungus diet, plant diet, diet-changed from plant to fungus, and under desiccation stress). High throughput sequencing generated 43 million reads with an average length of 100 bp. Assembly of the pooled read set generated 95,930 contigs, corresponding to 50,686 unigenes (mean length = 605 bp) with 45,244 alternate splice variants. Fifty-two percent of the unigenes (26,389) had homology to known genes or proteins in the NCBI non-redundant database, and 20,558 were assigned gene ontology (GO) terms based on these homologies by Blast2GO program (http://www.blast2go.com/b2ghome). Among the unigenes with blast hits, a large fraction were homologous to *Caenorhabditis* spp. and *Ascaris suum*. Two types of splice-leaders (SL) were identified: *A. fragariae* SL1:GGT TTA TTA ACC CAA GTT TGA G is very similar to *C. elegans* SL1, *A. fragariae* SL2:GGT TTA AAT ACC CAA ATT TGA G shares the same sequence as *Aphelenchus avenae* SL1c. Our results provide a comprehensive sequence resource for the study of the biology and physiology of *A. fragariae*.

REAL TIME INTERNET INVASIVE PEST INSECT AND/OR NEMATODE IDENTIFICATION. **Giblin-Davis<sup>1</sup>**, **Robin M., and A.L. Roda<sup>2</sup>**. <sup>1</sup>Fort Lauderdale Research and Education Center, University of Florida-IFAS, 3205 College Avenue, Fort Lauderdale, FL 33314-7719; and <sup>2</sup>USDA, APHIS, Plant Protection and Quarantine, Center for Plant Health Science and Technology (CPHST), ARS Subtropical Research Station, 13601 Old Cutler Road, Miami, FL 33158.

Early detection of potentially invasive pests is critical to avert significant economic and environmental damage that may result from their successful introduction and establishment in the United States. Once an invasive pest becomes established or spreads significantly, the cost to eradicate, suppress, or manage it can cost millions of dollars. Recent advances in affordable USB compliant digital microscope cameras with their own LED lighting systems and internet platforms for disseminating information in real time are creating the potential for training for insect or nematode pest identification in disparate locations. We chose the palm weevil genus *Rhynchophorus* as a test group of insects for assessing a real time internet pest identification training program in the greater Caribbean basin. Because one species in this genus of weevils, i.e. *Rhynchophorus palmarum*, is considered a major vector of the red ring nematode of palms, *Bursaphelenchus cocophilus*, we also assessed the possibility of real time nematode invasive pest training using the same internet platform but with slight modifications to the USB digital

microscope used. Preliminary local tests suggested that remote identification training using Rhynchophorus adults is possible with the U.S. government internet-based portal "FoodShield" which employs Adobe Connect software along with an open conference call line to reduce audio feedback issues. This interface allowed the "instructor" to show important identifying features of a pest organism to help distinguish it from similar native species. A training module was developed using easy to use keys with quality photographs of diagnostic characters for species of *Rhynchophorus* that were distributed with an observation kit (containing DinoCapture 2.0 image capture software, a DinoLite AD413TL digital microscope with enhanced working distance, a DinoLite MS35B microscope stand, and a DinoLite MS16C multi-positioning specimen holder) to remote participants along with number-coded but unidentified vouchers specimens of R. cruentatus, R. palmarum and R. ferrugineus prior to the training tests. The screen-sharing features of the portal allowed each test participant to project back images of diagnostic features of their unknowns for confirmation that they were correctly identifying their voucher specimens. As an additional test, we procured and evaluated a DinoLite AM7023 digital eyepiece camera (23 mm diam. with a 30 mm and C-mount adaptor) that was used in combination with a Zeiss student compound microscope using prepared slides of specimens of nematodes that were isolated or cultured from Rhynchophorus palmarum (i.e., Teratorhabditis palmarum, Acrostichus rhynchophori, Caenorhabditis angaria, Bursaphelenchus gerberae, and Bursaphelenchus cocophilus). This camera produced acceptable images for screen sharing and remote real time discussions about identifying features of these disparate nematodes from the same insect host as long as the microscope optics were good.

#### EFFICACY OF ORGANIC SOIL AMENDMENTS FOR CONTROL OF SOYBEAN CYST NEMATODE IN GREEN-HOUSE EXPERIMENTS. **Grabau**, **Zane and S.Y. Chen.** University of Minnesota Southern Research and Outreach Center, 35838 120<sup>th</sup> St, Waseca, MN 56093.

Soybean cyst nematode (SCN), Heterodera glycines, is a major yield-limiting pathogen of soybean. Some organic soil amendments are known to suppress nematode populations through direct or indirect action. A greenhouse experiment was conducted to screen for organic soil amendments to manage SCN. Ten soil amendments at various application rates (a total of 19 treatments) were tested: Corn (Zea mays) condensed distillers solubles (CDS), ash of combusted CDS, ash of combusted turkey manure, marigold (Calendula officinalis) plant powder, marigold plant, canola (Brassica napus) meal, field pennycress (Thlaspi arvense) seed powder, field pennycress plant, spring camelina (Camelina sativa) plant, and Cuphea (interspecies hybrid 'MNPSR23') plant. Soil amendments were incorporated into pots of SCN-free field soil, inoculated with SCN eggs, and soybeans were planted in the soil. SCN population density and plant height were measured at 40 days after planting (DAP) and 70 DAP, corresponding to about one and two SCN reproductive cycles, respectively. Plant shoot dry mass was also recorded at 70 DAP. The experiment was conducted twice, once in spring 2011 and again in winter 2012. In the absence of significant interactions, the results of the two experiments at 40 DAP were combined. Soil amendment treatment significantly affected SCN population at 40 DAP (P < 0.0001). Marigold plant at application rate of 2.9% (amendment:soil, w:w; same below), pennycress seed powder at 0.5%, canola meal at 1%, and CDS at 4.3% were most effective with SCN egg population reductions of 46.6%, 46.7%, 73.2%, and 73.3% compared to non-amended control, respectively. Soil amendment treatment significantly affected plant height at 40 DAP (P < 0.0001) resulting in similar or reduced plant height compared to control. Experiments were analyzed separately at 70 DAP due to significant interactions between experiment and treatment effects. For Experiment 1, soil amendment treatment significantly affected SCN population (P = 0.0019), plant height (P = 0.00190.0191), and plant mass (P = 0.0002) at 70 DAP. Only canola meal at 1% significantly reduced SCN with 70% reduction compared to control. CDS at 4.3%, ash of CDS at 0.2%, and ash of turkey manure at 1% increased plant mass by 42%, 34% and 28%, respectively, although CDS at 4.3% also reduced plant height by 22%. In Experiment 2, there were no significant effects of soil amendment treatment on SCN population, plant height, or plant mass. These results show some organic soil amendments effectively reduce SCN population after one generation, but are not consistently effective after two generations. The amendments may have had short-term nematicidal action that dissipated over time. High initial SCN population size seems to dilute effects of amendment after two generations as overall SCN population across treatments at 40 DAP was higher in Experiment 2 than Experiment 1 and amendment effects were absent at 70 DAP for Experiment 2.

# MULTITROPHIC INTERACTIONS INVOLVING ENTOMOPATHOGENIC NEMATODES APPLIED AGAINST PINE WEEVILS IN A FOREST ECOSYSTEM. Griffin, Christine T.<sup>1</sup>, A.M. Dillon<sup>2</sup>, C.D. Harvey<sup>1</sup> and C.D. Williams<sup>1</sup>. <sup>1</sup>Department of Biology, National University of Ireland Maynooth, Ireland; and <sup>2</sup>Coillte Teoranta, Newtownmountkennedy, County Wicklow, Ireland.

Entomopathogenic nematodes can be successful against insects in cryptic habitats, including pine weevils (*Hylobius* spp.) which are serious pests of forestry in northern temperate regions. Pine weevils breed in stumps of felled coniferous trees, and emerging adults feed on newly planted seedlings. In Europe, populations of *Hylobius abietis* are suppressed using nematodes applied around tree stumps to target developing weevils. Here we explore intraguild and trophic interactions in this below-ground forest ecosystem. First we examine factors influencing the success of nematodes against the target, using a meta-analysis of 22 field trials. The analysis showed that nematode species and soil type affected success to a much greater extent than tree species (*Pinus* spp. or *Picea sitchensis*). *Heterorhabditis downesi* was superior to *Steinernema carpocapsae*, and efficacy of

#### 464 Journal of Nematology, Volume 44, No. 4, December 2012

both nematode species was greater in highly organic peat than in mineral soils. However, S. carpocapsae performed surprisingly well for an ambush forager, locating weevils inside tree roots at depths of more than 40 cm in soil. Laboratory studies show that S. carpocapsae may use the tree roots as "route-ways" enabling them to find weevils deep in soil. While there was no evidence that host density affected nematode efficacy, it had a positive impact on nematode persistence after 48 months, suggesting that the infective juveniles recovered at this time had recycled in hosts. Clearfelled coniferous forests represent a semi-natural habitat. At the time when nematodes are applied (1-2 years after felling), stumps have been colonised by diverse invertebrates and microbes. Neither H. downesi nor S.carpocapsae adversely affected numbers, diversity or species composition of non-target beetle species emerging from stumps. A non-target insect of particular concern is the native parasitoid of pine weevil larvae, Bracon hylobii. In the laboratory, nematodes can kill parasitoid larvae and adults and can compete with them for hosts, but in field trials applied nematodes and native populations of B. hylobii had additive suppressive effects on populations of H. abietis. Native wood-colonising and/or entomopathogenic fungi may affect pine weevils in stumps, and the application of selected fungi, either native or exotic, is considered an alternative or additional control measure. Simultaneous application of entomopathogenic fungi and nematodes showed, at best, additive effects on pine weevil. In laboratory experiments, colonization of wood with the saprotrophic fungus Phlebiopsis gigantea affected the numbers of nematodes invading pine weevils, with the nature of the effect dependent on the nematode species. In the field, success of nematodes was reduced when stumps were colonised by augmentation with a native saprotrophic fungus, while success of B. hylobii was enhanced, suggesting that the fungus affected the outcome of competition between nematode and parasitoid. Exploration of the interactions between applied agents and the community of pests, their antagonists and non-target organisms can help develop effective and environmentally secure means of pest control.

EFFECT OF DIFFERENT CHEMICAL SEED TREATMENTS ON SUGAR BEET CYST NEMATODE. **Hafez, Saad L.<sup>1</sup>**, **M. P. Pudasaini<sup>1</sup>, K. Luff<sup>2</sup>, and R. Portenier<sup>1</sup>.** <sup>1</sup>University of Idaho, Parma Research and Extension Center, 29603 U of I Ln, Parma, ID 83660; and <sup>2</sup>Bayer CropScience3554 East 4000 North, Kimberly, ID 83341.

A field experiment was conducted at the University of Idaho Research and Extension Center in Parma, Idaho to study the efficacy of several seed, soil and foliar applied insecticide/ nematicide treatments on sugar beet cyst nematode. The fourteentreatment trial was established as a random complete block design with five replications. Sugar beets were planted into a silt loam soil with initial sugar beet cyst nematode populations ranging between 1 and 38 viable cysts per 500 cc of soil with an average of 10. Temik was applied at planting and again sequentially as a post-emergence side-dress placement. Poncho Beta FS 453, Poncho 600 FS, Votivo 240 FS, Poncho Votivo and Trilex Flowable were applied alone and/or in various seed treatment combinations. Movento 240 SC was applied to sugar beet foliage using a CO<sub>2</sub> handheld plot sprayer at 54 and 75 days after planting or starting at the two-leaf stage of development and then sequentially on a two week interval for a total of four applications. MSO @ 0.25% v/v was tank mixed with all Movento treatments. Data demonstrates that most treatments increased sugar beet yield by 12.5 to 23.3 percent as compared to the untreated control. The Temik standard resulted in a 23.3 percent increase and was the top yielding treatment. Combining Poncho 600 FS with Votivo 240 FS at1 and 10 miu/seed increased yield by approximately 22%. Poncho Beta FS 453 in combination with Votivo 240 FS at 1, 5 and 10 miu/seed increased yield by approximately 17%. Four applications of Movento starting at the two-leaf stage and repeated on a two week interval provided significant yield benefit (17%). Movento following Poncho Beta FS 453 or Poncho 600 FS + Votivo 240 FS did not provide added yield benefit as compared to either product alone. In conclusion, various treatments of Poncho Beta FS 456, Poncho 600 FS, Votivo 240 FS, and Movento offer sugar beet cyst nematode activity similar to Temik and should be further developed as nematode management tools in sugar beets.

EFFECT OF MCW-2 ON POTATO YIELD AND NEMATODE ASSOCIATED WITH POTATO IN IDAHO. **Hafez, Saad L.**<sup>1</sup>, **M. P. Pudasaini**<sup>1</sup>, **C. Schiller**<sup>2</sup>, and **R. Portenier**<sup>1</sup>. <sup>1</sup>University of Idaho, Parma Research and Extension Center, 29603 U of I Ln, Parma, ID 83660; and <sup>2</sup>Mana Inc., 1365 Sugarloaf Drive, Alamo, CA 94507.

A study on the effect of MCW-2 (fluensulfone), a new non-fumigant systemic nematicide (MANA Inc.), against Columbia root-knot nematode (CRKN) and Stubby root nematode (SRN) in two different fields and against Root lesion nematode (RLN) in greenhouse on potato was conducted. Ranger Russet potato seed pieces were planted. In the CRKN experiment, Mocap as a standard was pre-plant incorporated. MCW-2 @ 0.89 gal/A with Mocap was pre-plant applied and incorporated into 8-10 inch deep using a rototiller. MCW-2 @ 0.89 gal/A with Mocap was pre-plant applied. MCW-2 @ 0.89 gal/A at pre-plant followed by Vydate starting at 1440 DD and at two weeks intervals for a total of 7 applications was applied. In the SRN experiment, Temik as a standard was applied in furrow at planting. MCW-2 @ 0.89 gal/A was pre-plant broadcasted and incorporated 8-10 inch deep using a rototiller. MCW-2 @ 089 gal/A at pre-pant followed by Vydate at two weeks intervals for a total of 5 applications was applied. In the RLN experiment, Vydate as a standard at planting and at two weeks intervals for a total of 6 applications was applied. MCW-2 @ 1.78 and 3.57 lb ai/A were pre-plant applied. Vydate at planting followed by MCW-2 @ 1.78 lb ai/A was applied at four weeks after germination. Untreated control was included in all experiments. Potato tubers were hand-harvested and graded to determine total, clean or marketable, and infected yield. Results showed that, in the CRKN experiment, the total and clean yield of potato was increased in all treatments compared to

untreated control. MCW-2 alone resulted in higher total and clean yield with lower percent of infected tubers compared to untreated control, however, MCW-2 followed by seven foliar applications of Vydate provided the highest total and clean yield with the lowest percent of infected tubers compared to MCW-2 alone and other treatments. MCW-2 + Vydate had 19% infected tuber compared to 35-76% in other treatments and 94% in untreated control. In the SRN experiment, the total yields of potatoes were increased while total infected tuber yield was decreased in all treatments compared to untreated control. The MCW-2 alone and MCW-2 +Vydate did not show any difference in total tuber yield. However, MCW-2 +Vydate had lower infected yield (21%) compared to MCW-2 alone (47%). In the RLN experiment, Vydate applied at planting followed by MCW-2 four weeks after germination had decreased soil populations of lesion nematode and increased total potato yield compared to MCW-2 alone and untreated control. In conclusion, application of MCW-2 with Vydate seems to be effective for the management of potato nematodes in Idaho which results lower infected yield with higher clean and marketable yield.

CHEMICAL MANAGEMENT OF ROOT LESION NEMATODE (*PRATYLENCHUS THORNEI*) IN ONION. **Hafez, Saad L.<sup>1</sup>, M.P. Pudasaini<sup>1</sup>, K. Luff<sup>2</sup>, C. Schiller<sup>3</sup>, and R. Portenier<sup>1</sup>.** <sup>1</sup>University of Idaho, Parma Research and Extension Center, 29603 U of I Ln, Parma, ID 83660; <sup>2</sup>Bayer CropScience, 3554 East 4000 North, Kimberly, ID 83341; and <sup>3</sup>Mana Inc., 1365 Sugarloaf Drive, Alamo, CA 94507.

Two separate experiments were conducted at Parma, Idaho to investigate chemical management of root lesion nematode in onions. Furrow irrigated onions were planted in a silt loam soil with an average pre-plant nematode population of 2,887/500 cc soil. In the first experiment Admire was applied in front of the planting shoe in a four inch band over the seed row. Sepresto was applied as a seed treatment. Both Admire and Sepresto were followed by broadcast foliar applications of Movento @ 5 oz/A + MSO beginning when the onions had sufficient leaf area (six weeks after planting) and again one week later. Admire and Sepresto treatments were also followed by Movento @ 1.66 oz/A + MSO concentrated in a six inch band for an effective treated acre rate of 5 oz. Banded treatments were also initiated six weeks after planting and continued weekly for a total of eight applications. In the second experiment, MCW-2 (fluensulfone), a new non-fumigant systemic nematicide (MANA Inc.), was tested at four rates; 0.45, 0.67, 0.89, and 1.78 gal/A. MCW-2 was pre-plant broadcast on the soil surface using a handheld  $CO_2$  plot sprayer and incorporated to a depth of two inches using a spike harrow. A Vydate L standard was broadcast on the foliage @ 1 gal/A beginning six weeks after planting and was repeated every two weeks for a total of six applications in both experiments. Total onion yield and the weight of cull, medium, jumbo, and colossal size categories were determined at harvest. In the first experiment, all treatments except Admire applied alone increased total yield by 15% to 70% as compared to the untreated control and generally produced greater yield than the Vydate L standard. While treatments of Admire followed by Movento increased total yield by 15% to 42% as compared to the untreated control, these plots yielded less than treatments of Sepresto followed Movento, which increased yield by 54% to 70 %. It should be noted that Admire is typically recommended as an in-furrow spray rather than the over-row band utilized in this study. Movento applications following Admire or Sepresto generally shifted bulb size to larger categories. In the second experiment MCW-2 @ 0.45 gal/A increased total onion yield by 26% as compared to the untreated control. Jumbo and colossal bulb yields were also increased as compared to the control or the Vydate L standard. Data from these studies demonstrates that Admire or Sepresto followed by Movento or lower rates of MCW-2 can be effective components of a lesion nematode management program for onions.

## DAGGER NEMATODES AND PERENNIAL FRUIT CROPS, **Halbrendt**, John<sup>1</sup>, and J.A LaMondia<sup>2</sup>. <sup>1</sup>Penn State Fruit Research and Extension Center, P.O. Box 330, Biglerville, PA 17307; and <sup>2</sup>Valley Laboratory, 153 Cook Hill Road, Windsor, CT 06095.

The American dagger nematode (Xiphinema americanum sensu lato) is a weak parasite that causes little direct damage to perennial crops. Nevertheless, the nematode is considered a serious economic pest to the fruit industry because it is an efficient vector of Tomato Ring Spot Virus (ToRSV) which causes disease in apple, peach, plum, apricot, cherry, grape and cane fruit. Crops infected with ToRSV suffer a loss of vigor, produce unmarketable fruit and/or have a shortened productive life. The nematode can also transmit Tobacco Ring Spot Virus (TbRSV) that infects blueberry and causes similar problems. Dagger nematodes are found throughout the fruit growing regions of the mid-Atlantic states and have a broad host range. Similarly, ToRSV infects many different plants and common weeds may serve as virus reservoirs. Due to the prevalence of ToRSV and dagger nematodes, many orchards and vineyards are at risk of infection and require effective control measures to remain healthy and productive. Soil fumigation is an effective tool for the remediation of dagger nematode infested sites and is considered by many growers to be the most efficient method of preparing orchard or vineyard land for replanting. In recent years some fumigants have been banned and products that remain available must adhere to new stringent regulations governing application. The effect of the new guidelines is to add to the cost of soil fumigation or in some cases prohibits its use. As a consequence, some pesticide companies in the Northeast have discontinued custom fumigation services and it has become increasingly difficult, expensive and frustrating for fruit growers to source their soil fumigation needs. An alternative approach for managing the dagger nematode / ToRSV problem with cultural practices has been developed and is gaining acceptance among growers. The goal is to use a pre-plant regimen of biofumigation, rotation crops and herbicides to simultaneously suppress dagger nematodes and eliminate virus reservoirs. After planting, the focus centers on preventing the reintroduction of

#### 466 Journal of Nematology, Volume 44, No. 4, December 2012

virus through an aggressive weed management program. Dagger nematode populations will eventually increase but the crop will remain healthy if the virus is not present. Each method of nematode / virus management has advantages and disadvantages that a grower must consider as he/she plans for future plantings. Soil fumigation is quick and efficient but may be difficult to arrange and is expensive. Cultural management is less costly but may delay planting by two years. However, as an additional benefit, rotation crops have been shown to improve soil conditions and improve the growth of young trees.

## HOST SEEKING AND THE EVOLUTION OF OLFACTORY BEHAVIOR IN PARASITIC NEMATODES. Hallem, Elissa A., M.L. Castelletto, K.E. Chaisson, M.L. Guillermin, and J. Lee. Department of Microbiology, Immunology, and Molecular Genetics, University of California, Los Angeles, Los Angeles, CA 90095.

How do skin-penetrating parasitic nematodes locate hosts to infect? We are addressing this question using the mammalianparasitic nematodes Nippostrongylus brasiliensis, Strongyloides ratti, and Parastrongyloides trichosuri as model systems. N. brasiliensis is closely related to the human-parasitic hookworms Ancylostoma duodenale and Necator americanus, while S. ratti and P. trichosuri are closely related to the devastating human-parasitic threadworm S. stercoralis. We are also using the entomopathogenic nematodes (EPNs) Heterorhabditis bacteriophora and Steinernema carpocapsae, as well as Caenorhabditis elegans, as comparative models for the mammalian-parasitic nematodes. We are examining the host-seeking behaviors of parasitic infective juveniles (IJs) in response to the universal host cue carbon dioxide ( $CO_2$ ) as well as large panels of host-derived odorants. We previously showed that EPN IJs and C. elegans dauers, which are analogous life stages, are strongly attracted to CO<sub>2</sub>. Moreover, attraction of EPNs to potential insect hosts is dramatically reduced in the absence of CO<sub>2</sub>. By contrast, we find that mammalian-parasitic IJs are repelled by CO<sub>2</sub> alone, suggesting they rely on host-specific odors for host location. Both EPNs and mammalian-parasitic nematodes respond to a wide range of chemically diverse odorants. EPNs respond strongly to a number of insect-derived odorants, while mammalian-parasitic nematodes respond strongly to a number of human skin odorants, including some that are also attractive for anthropophilic mosquitoes. Overall, parasite odor response profiles reflect host range: when the parasites are clustered based on their olfactory responses, species with similar host preferences cluster together regardless of their phylogenetic distance. This suggests a critical role for olfaction in niche partitioning and the evolution of host range among parasitic nematodes. We are now investigating the neural basis of hostseeking behavior in parasitic IJs. We are identifying the sensory neurons that mediate responses to host-derived odorants and live hosts in mammalian-parasitic IJs. We will perform a functional analysis of these neurons in the parasites, as well as the analogous neurons in C. elegans dauers, using calcium imaging. These experiments will provide insight into how parasitic nervous systems have evolved to enable parasite-specific behaviors.

DIFFERENTIATION OF *MELOIDOGYNE FLORIDENSIS* FROM OTHER ROOT-KNOT NEMATODES IN FLORIDA USING MTDNA-RFLP. **Han, Hyerim<sup>1</sup>**, **J.A. Brito<sup>2</sup>**, and **D.W. Dickson<sup>3</sup>**. <sup>1</sup>Division of Forest Insect Pests and Diseases, Korea Forest Research Institute, Seoul, Republic of Korea, 130-712; <sup>2</sup>Division of Plant Industry, DPI, Gainesville, FL 32614; and <sup>3</sup>Department of Entomology and Nematology, University of Florida, Gainesville, FL 32611.

Meloidogyne floridensis, which is known to occur only in the USA, was first reported infecting root-knot nematode resistant peach rootstocks cvs. Nemaguard and Okinawa in 1966 in Gainesville, Florida. In 1991, this nematode was also reported reproducing on two newly developed root-knot nematode rootstocks, Nemared and Guardian. Morphologically this species resembles *M. incognita*, which it was initially described as being, but it also resembles *M. christiei* and *M. graminicola*, both which occur in Florida, and *M. hispanica*. Our objective was to develop a molecular identification protocol to distinguish M. floridensis from other root-knot nematode species occurring in Florida that will be reliable, fast and inexpensive. mtDNA-RFLP was applied using five isolates of *M. floridensis*, four of *M. arenaria*, and one isolate each of *M. christiei*, *M. enterolobii*, M. graminis, M. graminicola, M. hapla, M. incognita, M. javanica, and M. partityla. For the PCR, nematode genomic DNA was extracted from multiple single females using a Qiagen DNeasy Kit (Qiagen, Valencia, CA) and mtDNA was amplified using C2F3/1108 primers set at 58 °C annealing temperature. The PCR products were 1.2 kb for both *M. floridensis* and *M. arenaria*, and 1.7 kb for M. javanica and M. incognita. M. christiei and M. enterolobii produced fragment of ca. 540 bp and 700 bp, respectively, whereas M. graminis, M. graminicola, M. hapla and M. partityla produced a fragment of ca. 530 bp. The mtDNA was useful for distinguishing *M. floridensis* from all root-knot nematode species except *M. arenaria*. For further discrimination of these two nematode species a RFLP analysis was performed by digesting the amplified mtDNA product, 1.2 kb, with Hinf I restriction endonuclease. Distinct restriction patterns for both species were observed. These results show that mtDNA-RFLP using Hinf I clearly separates M. floridensis from M. arenaria.

## THE USDA NEMATODE COLLECTION AND ITS DATABASE: VITAL ASSETS FOR SYSTEMATICS RESEARCH AND IDENTIFICATION. Handoo, Zafar, J.D. Mowery, D.J. Chitwood, and L.K. Carta. USDA ARS Nematology Laboratory, Beltsville, MD, 20705.

The USDA Nematode Collection, one of the largest and most comprehensive nematode repositories in the world, continues to serve as a vitally important resource for nematode research and identifications. The Collection, assembled from worldwide sources, includes several constituent divisions that collectively consist of over 44,400 slides and vials stored in fire-proof safes. This resource serves as a major asset for taxonomic research and is used for a wide variety of scientific and regulatory purposes. Established in 1960 by Dr. A. Morgan Golden, the Collection includes many free-living, insectparasitic, marine and freshwater nematodes, although plant-parasitic nematodes are the most heavily represented. The oldest slide in the Collection was prepared by Nathan A. Cobb in 1890; the thousands of specimens collected from other pioneers include potentially extinct species isolated from the former Arlington Farm in Virginia. The constituent divisions of the Collection are the Type Collection with 6,709 slides and 613 vials, the General Collection with 18,551 slides and 7,095 vials, the Thorne Collection with 6,600 slides, the Steiner Mermithid Collection with 2,303 slides, the Mass Collection with 1,035 slides and 1,095 vials, the Gates Collection with 356 slides, and a Demonstration Collection of 87 museum jars. All depositions are entered into a database in which over 38,500 entries are searchable and available to the public at http://nt. ars-grin.gov/nematodes/search.cfm. Essential data on nematode host and distribution are recorded for each species, and by thoroughly studying database records important information and relationships between these nematodes and their environment can be gained. This database is regularly visited by scientists and regulatory agencies around the world and in the first three months of 2012 there were more than 1200 visits to the website. The unique specimens and data in the Collection have been used to resolve billion-dollar issues involving agricultural trade. Scientists around the world have regularly incorporated specimens in the Collection into their research. Along with the publicly available database records, many specimens are available for loan for limited periods to scientists in trusted organizations. Depositions made by scientists and other workers around the world are always welcomed and encouraged. The overall outcomes of Collection activities include the facilitation of agricultural trade, the continued protection of agriculture against economically dangerous invasive species, and the advancement of nematode taxonomy and scientific research.

RELATIONSHIPS AMONG SPECIES CLOSELY RELATED TO *HOPLOLAIMUS GALEATUS* IN THE UNITED STATES. **Holguín, Claudia M.<sup>1</sup>, P. Agudelo<sup>1</sup>, and J.D. Mueller<sup>2</sup>.** <sup>1</sup>School of Agricultural, Forest, and Environmental Sciences, Clemson University, Clemson, SC 29634; and <sup>2</sup>Edisto Research and Education Center, Clemson University, Blackville, SC 29817.

Lance nematodes, *Hoplolaimus galeatus*, are ecto-endoparasitic nematodes widely distributed in the United States that affect important agricultural crops. Despite their economic importance, little is known about their genetic variability. Furthermore, several species of *Hoplolaimus* are morphologically similar to *H. galeatus*, sharing characters such as four incisures in the lateral lines, three esophageal gland nuclei, a hemizonid anterior to the excretory pore, and the presence of abundant males. We studied the genetic variability within *H. galeatus* and the phylogenetic relationships with closely related species isolated from different crops and locations in the United States. We used morphology, biology, and sequences of the internal transcribed spacer 1 (*ITS1*) and cytochrome oxidase c subunit I (*COI*) for comparisons among the *Hoplolaimus* species isolated. The phylogenetic analysis shows the morphologically distinct *H. columbus* to be clearly separated from the species morphologically similar to *H. galeatus*. Within the latter group, *H. galeatus*, *H. magnistylus*, *H. stephanus*, *H. concaudajuvencus* and two undescribed species form separate clades. A better delimitation of these lance species will allow an improved interpretation of the published data on the distribution and the host/parasite relationships and will help elucidate the ecological and population genetic processes affecting establishment of lance nematodes.

EVALUATING SOYBEAN RESPONSES TO ROOT-KNOT NEMATODE USING VIRUS-INDUCED GENE SI-LENCING. **Hou, Jing<sup>1</sup>, S. Ghabrial<sup>2</sup>, A. Kachroo<sup>2</sup>, and P. Agudelo<sup>1</sup>.** <sup>1</sup>School of Agricultural, Forest, and Environmental Sciences, Clemson University, Clemson, SC, 29634; and <sup>2</sup>Department of Plant Pathology, University of Kentucky, Lexington, KY 40546.

*Meloidogyne incognita* is one of the most economically damaging plant-parasitic nematodes on soybean. A series of plant defense proteins have been shown to be activated upon infection by root-knot nematodes. The expression of these plant defense proteins can be regulated by phytohormones such as salicylic acid (SA). In many plants, SA is generally linked with the defense against biotrophic pathogens. The objective of this study was to observe the effect on root-knot nematode reproduction of the altered expression of genes that affect the levels of salicylic acid in the plant. We used a virus-induced gene silencing (VIGS) system, using a bean pod mottle virus (BPMV) vector, to target select enzymes in the phenylpropanoid and fatty acid pathways. After silencing and/or overexpression, the effects on soybean response to root-knot nematode were evaluated. Soybean plants of a susceptible (Hutcheson) and a resistant (Perrin) soybean cultivar were inoculated with the virus alone, the virus constructs, and buffer alone (mock inoculation). Forty-five days after inoculation, galling and egg masses per root were evaluated on the plants. An effect of treatments was observed on the susceptible variety, but not on the resistant. On Hutcheson, higher nematode reproduction and galling were observed on the plants infected with the virus alone and the controls. BPMV infection increased SA accumulation in the plant, so empty vector-inoculated plants had more SA content than untreated plants. The tested constructs silenced key enzymes and reduced SA levels probably by shutting down one pathway for SA biosynthesis. In other words, high SA was correlated with increased susceptibility to the nematode in the susceptible host.

NEMATODES ASSOCIATED WITH AQUATIC PLANTS. Huang, Sih-Ying, Peichen Chen, and Tung-Tsuan Tsay. Dept. of Plant Pathology, National Chung Hsing University, 250 Kuo-Kuang Rd., Taichung 402, Taiwan.

Nematodes associated with aquatic plants were rarely studied. The aim of this study is to survey the free-living, predatory or plant-parasitic nematodes associated with commercial submerged aquatic plants. Forty species of dicotylendonous aquatic plants from 20 families, and 36 species of monocots from 5 families were investigated. Seven families of free-living nematodes were found to associate with aquatic dicots in the families of Chronogasteridae, Cryptonchidae, Diplopeltidae, Leptolaimidae, Mesorhabditidae, Rhabditidae and Tripylidae. The predatory nematodes in the families of Actinolaimidae, Anatonchidae, Cyanotholaimidae and Dorylaimidae were attained from aquatic dicots. Only one genus of plant-parasitic nematodes, identified as Aphelenchoides, was isolated from nine families of aquatic dicots. From monocotylendonous aquatic plants, free living nematodes in the families of Cephalobidae, Chronogasteridae, Cryptonchidae, Leptopaimidae, Mesorhabditidae, Panagrolaimidae, Plectidae, Rhabditidae, Rhabdolaimidae and Tripylidae were detected. Nine families of predatory nematodes in the families of Actinolaimidae, Aporcelaimidae, Belondiridae, Dorylaimidae, Metateratocephalidae, Monhysteridae, Mononchidae, Mylonchulidae and Tylencholaimellidae were found from aquatic monocots. Parasitic nematodes belonging to Aphelenchoides, Meloidogyne and Hirschmanniella were isolated from Araceae, Hydrocharitaceae and Najadaceae of aquatic monocots. Chronogateridae of free-living nematodes and Dorylaimidae of predatory nematodes were the two dominant families found in this survey. Many second-stage juveniles of Meloidogyne sp. were recovered from economically important anubias aquatic plants with galling symptoms on the roots. When mitochondria DNA of five singlefemale derived Meloidogyne populations were amplified, all yielded an approximately 1.7 kb fragment. The results of SCAR-PCR indicated that the root knot nematodes were either *M. arenaria* or *M. incognita*.

## HETEROPLASMY AND GENETIC STRUCTURE IN *MELOIDOGYNE CHITWOODI* FROM THE UNITED STATES. **Humphreys, Danny A. and A.A. Elling.** Dept. of Plant Pathology, Washington State University, P.O. Box 646430, Pullman, WA 99164.

Mitochondrial DNA (mtDNA) is thought to evolve faster in Nematoda than in other taxa; the resulting polymorphisms can be useful as diagnostic markers and enable exploration of the genetic structure of root-knot nematode populations. The current knowledge about mtDNA polymorphisms and the genetic structure in Meloidogyne, especially in minor species such as M. chitwoodi is very limited. The objective of this study was to investigate the genetic structure of four M. chitwoodi isolates, representing all races and pathotypes currently known in the United States, by studying mtDNA variability. Additionally, we analyzed whether M. chitwoodi shows heteroplasmy (i.e., the presence of more than one mtDNA genome). To study mtDNA polymorphisms, we amplified a region between the 3' end of the cytochrome oxidase subunit II (COII) and 16S rRNA mitochondrial genes using primers 1108 and C2F3. PCR amplicons from eight individual second-stage juveniles of each M. chitwoodi isolate were cloned and five clones for each of the eight individuals (160 clones total) were sequenced bidirectionally. We found that 94% of the individual nematodes we typed had three or more mitochondrial haplotypes, and 34% showed differences between all of the five sequences we analyzed for each nematode. Strikingly, none of the individuals we surveyed was homoplasmic. We detected 99 haplotypes among the 160 sequences analyzed (representing 32 individuals), which suggests a high level of heteroplasmy in *M. chitwoodi*. AMOVA showed that the majority of the variation was within isolates (91%) and that the differentiation among the four isolates was low but significant (P < 0.001). Results from pairwise comparisons and  $S_{nn}$  analysis suggest that WAMC1 (race 1) is distinct from the other isolates based on mtDNA. Possible explanations for the observed low genetic structure between isolates are sexual reproduction of different races and pathotypes under field conditions or a common founder population that results in similar patterns of diversity. Haplotype diversity (h) and nucleotide diversity ( $\pi$ ) were similar in all four isolates, giving a high overall h and a relatively low overall  $\pi$ , which may be interpreted as possible signs of a recent demographic expansion. This conclusion is supported Tajima's D and Fu's  $F_{\rm S}$ neutrality tests, resulting in negative and statistically significant values (all P < 0.0001). Furthermore, a haplotype network analysis resulted in a star-like pattern surrounding a single dominant haplotype. These results are the first insights into the genetic structure of *M. chitwoodi* and set the stage for future phylogenetic and phylogeographic analyses to determine the origin of this nematode species.

## MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION OF *MELOIDOGYNE CHITWOODI* ISOLATES FROM THE UNITED STATES. **Humphreys, Danny A. and A.A. Elling.** Dept. of Plant Pathology, Washington State University, P.O. Box 646430, Pullman, WA 99164.

*Meloidogyne chitwoodi* is a severe problem in potatoes, where it decreases tuber quality and affects crop marketability. To prevent further spread, *M. chitwoodi* has been designated a quarantine pathogen by many countries. Intraspecific variation is not well characterized for *M. chitwoodi*, but is critical to avoid misidentification and to optimize management strategies. The objective of this study was to analyze the morphological and molecular variation of four *M. chitwoodi* isolates, representing all races and pathotypes currently known in the United States. Morphological characters of second-stage juveniles, adult females and males were analyzed by light microscopy and scanning electron microscopy (SEM). Contrary to the original species description, perineal patterns from females in the present study showed punctations in all isolates. Morphometric data

for all four isolates were generally in agreement with previous descriptions for *M. chitwoodi*. However, we observed high variation in vulva slit length and distance from vulva to anus in females. Despite statistically significant morphological variation among adult females from different isolates (MANOVA, P < 0.0001), morphometrics was not able to reliably distinguish *M. chitwoodi* isolates. In contrast to morphology, molecular traits that are determined by isozymes and nuclear ribosomal genes were stable across all isolates. Malate dehydrogenase, esterase and superoxide dismutase isozyme phenotypes of all isolates showed species-characteristic isozyme patterns for *M. chitwoodi*. Similarly, PCRs with the widely used diagnostic primers JMV and 194/195 resulted in species-characteristic products for all races and pathotypes studied here, which indicates that there is no intraspecific variability in size in nuclear ribosomal genes that could affect proper species identification of *M. chitwoodi*. This is an important finding, because it shows that these molecular markers are reliable enough to be used routinely in *M. chitwoodi* species identification regardless of isolate, race and pathotype designation or geographical origin.

### NEMATICIDE APPLICATION STRATEGIES TO CONTROL NEMATODES IN POTATO. Ingham, Russell, E. Dept. of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331-2902.

Successful nematode control often entails finding the right application method for the right nematicide. This concept can be documented using nematodes in potato as examples. Root-knot nematodes (RKN), Meloidogyne hapla and M. chitwoodi infect potato tubers and cause brown spots to form around the infection sites. These spots are considered quality defects for which there is low tolerance in domestic markets and no tolerance in export markets. Corky ringspot disease (CRS) produces necrotic arcs and rings in tuber tissues that are caused by Tobacco Rattle Virus vectored to tubers by stubby root-nematodes, Trichodorus and Paratrichodorus spp. Crops with excessive symptoms from RKN or CRS may be devalued or rejected. Acceptable control of RKN and CRS requires getting maximum performance from nematicides. A number of different approaches to incorporating ethoprop with water were unsuccessful while several methods of physical incorporation before planting controlled M. hapla damage to tubers. Similarly, application of metam sodium (MS) in irrigation water did not control CRS or RKN, whereas, deeper placement of MS with shank injection suppressed damage from both CRS and RKN under low to moderate disease pressure. Under heavier pressure, deeper placement and a higher rate were required for adequate control. Timing of nematicide application can also be critical. Oxamyl can be used successfully to control CRS and RKN but requires that initial applications are made early in the season. Without applications in-furrow at planting and/or at emergence, control of CRS and RKN with oxamyl is very difficult. Often the best application strategy is using two different nematicides together. 1,3-dichloropropene (1,3-D) is generally very effective for control of CRS and RKN but occasionally soil conditions are not ideal and some nematodes can escape treatment. Following 1,3-D with MS, ethoprop, or perhaps oxamyl can ensure maximum protection. A tank mix of MS and ethoprop injected at 15 and 30 cm is superior to either product alone or both products applied separately. Control is also improved with combinations of MS and oxamyl, aldicarb and oxamyl, or ethoprop and oxamyl. Design of effective application techniques may be almost as important as development of product chemistry. Nematcides currently being developed may not be as powerful as those that have been lost, so optimum application technology will be critical. New and creative application strategies may be required to achieve optimum performance.

HATCHING AND REPRODUCTION OF A NEW SPECIES OF *GLOBODERA* (*G. ELLINGTONAE*) FOUND NEAR POWELL BUTTE OREGON. Ingham, Russell<sup>1</sup>, I.A. Zasada<sup>2</sup>, D.A. Navarre<sup>3</sup>, D.R. Kroese<sup>2</sup>, A.B. Peetz<sup>2</sup>, M. Ballato<sup>2</sup>, and N.M. Wade<sup>1</sup>. <sup>1</sup>Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331, <sup>2</sup>USDA-ARS Horticultural Crops Research Unit, Corvallis, OR 97330, <sup>3</sup>USDA-ARS Vegetable and Forage Crop Research Unit, Prosser, WA 99350.

A new species of *Globodera* (*G. ellingtonae*) has been described from a research farm owned by Oregon State University near Powell Butte, OR. No information was known as to the host range of this nematode and densities of cysts are very low (<1/3 kg soil). Because *Globodera* eggs do not hatch readily unless exposed to root exudates from a plant host, immersing eggs in root diffusates from different plants is a potentially rapid way to test if particular plants may be hosts that requires few cysts. Eggs from cysts collected at Powell Butte were exposed to root diffusates from potato, tomato, seven *Brassica* species, five graminoid species, seven weed species, and two species of vetch, and hatch was recorded. Water was used as a control. Some hatch occurred in all treatments but was only appreciable in tomato and potato. In greenhouse experiments to investigate host range, potato and tomato were found to be hosts but barley, oats, and alfalfa were not. Reproduction on different potato varieties was compared in the greenhouse and results were expressed as reproduction factor (RF) = egg recovery/inoculum level. Averaged over two trials, Russet Burbank was the best host (RF = 55.5) and Desiree (34.1), Modoc (31.4), Umatilla (27.9), Norland (23.7), and Yukon Gold (15.1) were also good hosts. Egg numbers did not increase on Maris Piper (RF = 0.6), Atlantic (0.6) or Setina (0.4). In contrast, when reproduction on different potato varieties was evaluated in field microplots, RF values were lower for Russet Burbank (9.7), Modoc (4.8), and Yukon Gold (1.5) than in the greenhouse. This was attributed to the fact that potatoes in microplots died early due to frost. The pattern of susceptibility/resistance on these potato varieties resembles that of *G. rostochiensis*. Studies are planned to determine if *G. ellingtonae* is a potato pathogen. This nematode has not been found in any other fields in Oregon.

A PRELIMINARY SURVEY OF FIG-ASSOCIATED NEMATODES IN THE ASIAN SUBTROPICS. **Kanzaki, Natsumi<sup>1,2</sup>**, **R. Tanaka<sup>2</sup>, R.M. Giblin-Davis<sup>1</sup>, E.J. Ragsdale<sup>3</sup>, C.N. Nguyen<sup>4</sup>, H.-F. Li<sup>5</sup>, and Y.-C. Lan<sup>6</sup>. <sup>1</sup>Fort Lauderdale Research and Education Center, University of Florida-IFAS, 3205 College Avenue, Fort Lauderdale, FL 33314-7719; <sup>2</sup>Forest Pathology Laboratory, Forestry and Forest Product Research Institute, 1 Matsunosato, Tsukuba, Ibaraki, 305-8687, Japan; <sup>3</sup>Max Planck Institute for Developmental Biology, Department of Evolutionary Biology, Spemannstrasse 37, 72076 Tübingen, Germany; <sup>4</sup>Department of Nematology, IEBR-VAST, 18 Hoang Quoc Viet Rd., Hanoi, Vietnam; <sup>5</sup>Academia Sinica, Institute of Plant and Microbial Biology, No.128, Sec. 2, Academia Rd., Nangang Dist., Taipei 11529, Taiwan; and <sup>6</sup>Department of Leisure Resources and Green Industries, University of Kang Ning, 188, Sec. 5, An-Chung Rd., Annan District, Tainan City 70901, Taiwan.** 

Fig (Ficus syconia) - associated nematodes are diverse, with several different groups of nematodes including diplogastrids (Parasitodiplogaster, Teratodiplogaster, Mononchoides and some other undescribed genera), tylenchids (Ficotylus) and aphelenchoidids (Schistonchus) having been isolated from various subgroups of Ficus trees. Most of the fig-associated nematode studies have been conducted in North and Central America, Australia, Africa, and South Asia, yet knowledge of these nematodes in East Asia has been scarce. We conducted a preliminary survey of these nematodes in East Asian subtropical areas, i.e. from three fig species, Ficus variegata, F. septica, and F. auriculata in Japan, Taiwan, and Vietnam, respectively. F. variegata and F. septica were collected from Ishigaki and Iriomote Islands, Okinawa, Japan and from Orchid Island and the southern part of the main island of Taiwan. Specimens of F. auriculata were collected from Cuc Phuong National Park, Ninh Binh Province, Vietnam. Figs (B-C phases) were collected, brought back to the laboratory, and dissected under a stereomicroscope. Isolated nematodes were fixed in TAF for morphological comparisons or in DESS for combined morphological and molecular analyses. Some of the recovered nematodes were observed under a compound microscope prior to placement in nematode digestion buffer for molecular protocols. Although detailed molecular sequence analyses are still incomplete, several different morphotypes of nematodes were recorded from the examined fig species. An undescribed Teratodiplogaster sp. and two morphotypes of Schistonchus spp. were isolated from F. variegata and an unidentified rhabditid species and two morphotypes of Schistonchus were recovered from F. septica. These nematode species (morphotypes) were isolated from all four islands examined. Ficus auriculata did not harbor Teratodiplogaster, which is associated with other Sycomorus-group fig species, but at least two morphotypes of undescribed diplogastrids and a Schistonchus sp. were isolated from this fig species. Of these undescribed diplogastrids, one is morphologically similar to a species isolated from African figs. More surveys of fig-associated nematodes in the African and Asian tropics are necessary to elucidate the evolutionary history and diversity of fig-fig wasp-nematode associations.

#### NEMATODE DISPERSAL IS REGULATED BY AN EVALUTIONARY CONSERVED NEMATODE COMMU-NICATION SYSTEM. **Kaplan, Fatma<sup>1</sup>, H.T. Alborn<sup>1</sup>, S.H. von Reuss<sup>2</sup>, F.C. Schroeder<sup>2</sup>, and P.E. Teal<sup>1</sup>. <sup>1</sup>USDA-ARS, Gainesville, FL 32608; and <sup>2</sup>Boyce Thompson Institute, Cornell University, Ithaca, NY 14853.**

Root knot nematodes (*Meloidogyne* spp) are possibly the most economically important plant parasitic species and thus among the best-studied pest nematodes. Previously, it has been reported that *Meloidogyne* species prefer uninfected over infected roots when given a choice, thus they can detect and respond to signals related to infected roots. However, for Meloidogyne spp, Caenorhabditis elegans and most other nematode species, very little is known about signaling within and, even less so, in-between species. It is well known that during conditions unfavorable for normal growth and development C. elegans, produces stress resistant and food seeking dispersal larvae, called dauer. It has previously been shown that dauer formation is regulated by pheromones, called ascarosides. The dauer dispersal stage is analogous to the infective second stage juveniles (J2) of plant parasitic nematodes and infective juveniles (IJ)s of insect parasitic nematodes (EPN), eg. Steinernema feltiae. Regulation of dispersal behavior of dauer larvae has not been thoroughly investigated for C. elegans or any other nematode species. Similar to EPNs, the free living bacteriovor *Caenorhabditis briggsae* can infect and develop within insect larvae. This is also the case for C. elegans when raised with C. briggsae associated bacteria. These behavioral similarities suggested that *Caenorhabditis* spp. and phylogenetically related EPNs may utilize similar signaling molecules. Based on previously identified blends of ascarosides regulating mating behavior in C. elegans, we hypothesized that ascarosides might also be involved in regulation of dispersal behavior. Liquid chromatography-mass spectrometry analysis of C. elegans dauer conditioned media, which shows strong dispersing activity, revealed four known ascarosides (ascr#2, ascr#3, ascr#8, icas#9). A synthetic blend of these ascarosides at physiologically relevant concentrations dispersed C. elegans dauer in the presence of food and also caused dispersion of IJs of S. feltiae and J2s of plant parasitic Meloidogyne spp. Assay guided fractionation revealed structural analogs as major active components of the S. feltiae (ascr#9) and C. elegans (ascr#2) dispersal blends. Further analysis revealed ascr#9 in Steinernema spp. and Heterorhabditis spp. infected insect host cadavers. Ascaroside blends represent evolutionarily conserved, fundamentally important communication systems for nematodes from diverse habitats, and thus may provide sustainable means for control of parasitic nematodes.

LIPOPEPTIDE ANTIBIOTICS OF *BACILLUS SUBTILIS* IN THE MANAGEMENT OF ROOT-KNOT NEMATODE *MELOIDOGYNE INCOGNITA* IN NONI, *MORINDA CITRIFOLIA*. Kavitha, P.G.<sup>1</sup>, E.I. Jonathan<sup>2</sup> and S. Nakkeeran<sup>3</sup>. <sup>1</sup>Dept. of Nematology, <sup>2</sup>Center for Plant Protection Studies, and <sup>3</sup>Dept. of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore 641003, India.

The endospore-forming rhizobacterium Bacillus subtilis used as a model system for Gram-positive organisms produce more than two dozens of lipopeptide antibiotics, hydrolytic enzymes and other secondary metabolites. Biocontrol activity of Bacillus strains against multiple plant pathogens have been widely reported and well documented. Three families of Bacillus lipopeptides – surfactins, iturins and fengycins are mostly studied for their antagonistic activity against a wide range of phytopathogens, including bacteria, fungi and nematodes. Noni (Morinda citrifolia L.) considered as a panacea to cure many diseases is gaining popularity for its use as an alternative herbal medicine The root-knot nematode (Meloidogyne incognita) is one among the most damaging pathogens attacking a wide range of crops including noni. This nematode parasite destructive of noni causes severe root infections that reduce plant growth and ultimately lead to death of the plant. Six antagonistic endophytic strains of B. subtilis viz., Bs N 1, Bs N 3, Bs N 4, Bs N 7, Bs 5 and Bs N 11 were isolated from the noni fruits and plant tissues. An existing strain Bbv 57 isolated from banana proven for its nematicidal action was included in the study. The genomic DNA from the Bacillus strains was isolated using cetyl trimethyl ammonium bromide (CTAB) method and PCR amplified for antibiotic genes. Biosynthetic gene specific primers SUR3F and SUR3R amplified a 440 bp of surfactin gene for BsN 3, Bs 5 and Bbv 57. Iturin specific primers ITUD1F and ITUD1R amplified with the fragment size 648bp of iturin gene. Among all strains of Bacillus BS 5 and Bby 57 were amplified for iturin gene. B. subtilis strain BS5 with high surfactin and iturin activity has proven its ability to suppress root knot nematode in vitro. The study revealed that the crude antibiotic exerted maximum lethal effect on root knot nematode by inhibiting egg hatching and causing juvenile mortality. Juveniles (J1) inside the eggs were paralyzed and killed due to the antagonistic effect of the antibiotic and thereby proving its larvicidal action which is irreversible. SEM analysis of root knot nematode eggs after 48 hrs of treatment with antibiotic revealed the deposition of antibiotic over the nematode eggs which resulted in the death of the eggs due to its ovicidal action. Translocation and colonization potential of these effective endophytic strains of noni were studied by radio labelling method using the isotope  ${}^{32}P$  and trace analyzed by autoradiography. The success of B. subtilis is associated with the presence of the genes encoding surfactin and iturin synthesis and hence it can be a valuable candidate in the context of biological control for managing root knot nematode in noni.

ECOLOGY OF SOILS SUPPRESSIVE TO THE SOYBEAN CYST NEMATODE: I. EFFECT OF TILLAGE AND CROP-BIOCIDE TREATMENTS ON SOIL SUPPRESSIVENESS TO NEMATODE AND SOYBEAN YIELD. Kidane, Eyob<sup>1</sup>, Weiming Hu<sup>1</sup>, Senyu Chen<sup>1</sup>, and Deborah A. Neher<sup>2</sup>. <sup>1</sup>University of Minnesota Southern Research and Outreach Center, 120th Street, Waseca, MN 56093; and <sup>2</sup>Department of Plant & Soil Science, 63 Carrigan Drive, Burlington, VT 05405.

The soybean cyst nematode (SCN), Heterodera glycines, is the major pest problem in the corn-soybean production systems in the Midwest in the United States. Soil suppressive to SCN has been reported in a number of locations in the USA and other countries. A four-year field experiment was initiated in 2009 at a site in Minnesota to study the ecology of the soil suppressive to the SCN. The experiment was a split-plot design with no-till and conventional tillage as main plots, and five crop-biocide treatments as subplots with four replicates. The five crop-biocide treatments were 1) corn-soybean annual rotation without biocide, 2) soybean monoculture without biocide, 3) soybean monoculture with bactericide, streptomycin, 4) soybean monoculture with fungicide, captan, and 5) soybean monoculture with general biocide, formaldehyde. The soil suppressiveness to SCN was assessed by determining SCN egg population densities in the field at planting, midseason, and harvest, and by bioassay of the soil collected at the midseason in the greenhouse. Soybean yields were recorded. There was no effect of biocide treatment on the soybean yield, but conventional tillage resulted in 4% yield increase in 2010. Overall, treatment effects on nematode suppressiveness increased over the three years from 2009 to 2011. In 2009, no effect of biocide and tillage on SCN egg population density was detected. The general biocide treatment of soil in the field plots increased SCN egg population density in 2010 and 2011. In addition, the fungicide treatment resulted in greater egg population density than the no-biocide control and bactericide treatments in 2011. No effect of the bactericide treatment on the egg population density was observed at any sampling occasion over the three years. A slight increase of SCN egg population density by conventional tillage was observed in the midseason in 2010, but not any other sampling occasion. In the greenhouse test, treatment of soil with autoclave heating significantly increased the nematode population density in the potting soil by two months after planting. Mixing 10% of the untreated soil with the autoclaved soil resulted in lower SCN egg population density as compared with the autoclaved soil, indicating that the suppression of nematode population was biological and could be transferred. However the greenhouse bioassay failed to detect any effect of tillage and crop-biocide treatment on the soil suppressiveness to SCN.

ECOLOGY OF SOILS SUPPRESSIVE TO THE SOYBEAN CYST NEMATODE: II. EFFECT OF TILLAGE AND CROP-BIOCIDE TREATMENTS ON NEMATOPHAGOUS FUNGI. **Kidane, Eyob<sup>1</sup>**, **Weiming Hu<sup>1,2</sup>**, **Senyu Chen<sup>1</sup>**, **Xingzhong Liu<sup>2</sup>**, **and Deborah A. Neher<sup>3</sup>**. <sup>1</sup>University of Minnesota Southern Research and Outreach Center, 120th Street, Waseca, MN 56093; <sup>2</sup>State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, China; and <sup>3</sup>Department of Plant & Soil Science, 63 Carrigan Drive, Burlington, VT 05405.

The soybean cyst nematode (SCN), *Heterodera glycines*, is the major pest problem in the corn-soybean production system in the Midwest of the United States. Soil suppressive to SCN has been reported in a number of locations in the USA and other regions in the world. A four-year field experiment was initiated in 2009 at a site in Minnesota to study the ecology of SCNsuppressive soil. The experiment was a split-plot design with no-till and conventional tillage as main plots, and five cropbiocide treatments as subplots with four replicates. The five crop-biocide treatments were 1) corn-soybean annual rotation without biocide, 2) soybean monoculture without biocide, 3) soybean monoculture with bactericide, streptomycin, 4) soybean monoculture with fungicide, captan, and 5) soybean monoculture with general biocide, formaldehyde. Soil samples were taken from each plot at planting, midseason, and harvest to quantify percentage of SCN second-stage juveniles (J2) parasitized by fungi. Occurrences of trapping fungi were determined with the samples collected in midseason of 2009 and 2010, and spring and midseason of 2011. In addition, egg-parasitic index values (EPI, on 0-10 scale) were determined at midseason every year. A mean of 13.4% (0-50%, range, same below) of J2 individuals were parasitized by fungi over the three years. The non-host corn consistently lowered percentage of J2 individuals parasitized by fungi. There was no consistent pattern of biocide treatment effect on fungal parasitism of J2 across the sampling occasions over the three years. Application of formaldehyde reduced the percentage of J2 parasitized by fungi three weeks after application in 2010, but it was not significant at other sampling occasions. Tillage did not affect percentage of J2 parasitized by fungi. Of fungi isolated from J2 during 2009-2011, 72.3% were Hirsutella rhossiliensis, 11.9% H. minnesotensis, and 15.8% other species. Mean Most Probable Numbers (MPN) of Colony-forming Units (CFU) of trapping fungi in the midseason of 2009, midseason of 2010, spring and midseason of 2011were 1.56 (0-8.5), 4.11 (0-16.5), 1.7 (0-11) and 0.83 (0-3.9) per gram of dry soil, respectively. No treatment effect on MPN of trapping fungi was detected except that the numbers in spring 2011 was greater in no-till than conventional tillage treatments. Most common species of trapping fungi encountered were Arthrobotrys oligospora and Arthrobotrys conoides. Average EPI at midseason was low at 0.47 (0-1.65), 1.84 (0.32-4.32) and 1.06 (0.24-2.56) in 2009, 2010 and 2011, respectively. No treatment effect on EPI values was detected at any sampling date. This study suggests Hirsutella rhossiliensis was probably the most important fungus in suppressing the SCN population density at the site.

NEMATODE FAUNA IN DOKDO ISLAND OF KOREA. **Kim, Donggeun<sup>1</sup>, B.Y. Park<sup>2</sup>, and Y.H. Ryu<sup>1</sup>.** <sup>1</sup>Institute for Natural Products Research, Gyeongbuk Agr. Res. & Ext. Serv.; and <sup>2</sup>Crop Protion Division, Dept. of Agr. Biol., Nat. Aca. of Agr. Sci., RDA.

Nematode fauna in Dokdo island was investigated for two years between 2008 and 2009. Population density and biomass were 239,500 (26,000-836,000)/m<sup>2</sup> and 103.9 mg/m<sup>2</sup> (3.0-388.6), respectively. Total of 31 species of nematodes were identified: *Acrobeloides* is the most important genus with prominence value (PV) of 117.8, followed by Rhabditidae (PV=81.9), *Prismatolaimus* (PV=39.0), and *Aphelenchoides* (PV=31.4). Among feeding group, bacteriovorus nematode composed 77% of density and 62% of biomass followed by plant-parasitic and fungivorus nematodes. A species of *Pratylenchus* having distinctive tail terminus is under study as a new species. Biological indics, Shannon and Wiener index (2.46), Simpson's diversity index (0.86), species evenness (0.73), species richness (1.30) indicate that nematode fauna in Dokdo island is diverse and unique. This study may give a general idea of nematode fauna at the Dokdo island.

## SUSCEPTIBILITY OF SEVERAL WEEDS COMMON IN FLORIDA AGRICULTURAL PRODUCTION TO *MELOIDOGYNE* SPP. **Kokalis-Burelle, Nancy and E.N. Rosskopf.** USDA-ARS, U.S. Horticultural Research Lab, 2001 S. Rock Rd., Ft. Pierce, FL 34945.

Documenting shifts in existing and newly emerging weed populations, and how these changes affect nematode populations is required as vegetable and ornamental producers transition from broad-spectrum fumigants to more sustainable methods for nematode control. In a Florida field trial examining alternatives to fumigants for production of field-grown cut flowers, weeds were collected, identified, and evaluated for galling by root-knot nematodes (*Meloidogyne* spp.). Cheeseweed mallow (*Malva parviflora* L.) was determined to be infected with *Meloidogyne arenaria*, and greenhouse experiments confirmed and quantified the host status under controlled conditions. This was the first report of *M. parviflora* as a natural host of *M. arenaria*. In greenhouse trials to assess galling and egg production of three common root-knot nematode species, *M. incognita*, *M. arenaria*, and *M. javanica* on weeds common in Florida production, *Portulaca oleracea* (purslane), *Eleusine indica* (goosegrass), *Aeschynomene americana* (American jointvetch), *Solanum americanum* (American black nightshade), *Cyperus esculentus* (yellow nutsedge), and *Amaranthus retroflexus* (redroot pigweed) were evaluated. Although recommended as a cover crop in the southern U.S., *A. americana* was evaluated as a weed following a heavy volunteer infestation of an experimental field in southeastern Florida where galling was observed on roots. *Portulaca oleracea* and *A.*  *americana* roots supported the highest number of juveniles  $(J_2)$  of all three species of *Meloidogyne*, and had the highest number of eggs/g root for all three species. However, although *P. oleracea* supported very high numbers of nematode  $J_2$  in roots, galling was moderate to low for all three *Meloidogyne* species. In contrast, galling on *A. americana* was higher than for *P. oleracea* for all three species, and more representative of the numbers of  $J_2$  isolated from roots. Low levels of apparent galling combined with high egg production, increases the potential for *P. oleracea* to affect populations of these three root-knot nematode species to a degree that may not be immediately recognized. Also. *A. americana* may serve as an important host of the three species of root-knot nematode tested in southern regions of Florida.

# EFFECTS OF NUTRIENTS ON RENIFORM NEMATODE (*ROTYLENCHULUS RENIFORMIS*) PATHOGENICITY AND REPRODUCTION. Kularathna, Manjula<sup>1</sup>, C. Overstreet<sup>1</sup>, E.C. McGawley<sup>1</sup>, D.M. Xavier<sup>1</sup>, C.M. Martin<sup>1</sup>, and D.B. Burns<sup>2</sup>. <sup>1</sup>LSU Agricultural Center, Department of Plant Pathology and Crop Physiology, 302 Life Sciences Building, Baton Rouge, LA 70803; and <sup>2</sup>P.O. Box 438, St. Joseph, LA 71366.

The effects of soil nutrients on nematode pathogenicity vary among different nutrient/nematode/crop plant systems. Nutrients can increase or decrease pathogenicity. Current research in Louisiana is focused on reducing the impact of reniform nematode on cotton. The available nematode management practices are either inefficient, expensive, or cause environmental concerns. Therefore, attention is being given to finding alternative methods to offset nematode damage. Precision agriculture research has indicated that soil texture is related to reniform nematode damage and response to nematicide application. In addition, low nutrient availability is associated with high nematode pathogenicity and soil texture. This finding suggests the possibility that selective nutrient management could provide an alternative to nematicide application to reduce reniform damage in cotton. Greenhouse and field studies were conducted to determine the effects of different nutrients on reniform nematode (Rotylenchulus reniformis) pathogenicity and reproduction on cotton. In the greenhouse study, combinations of high and low phosphorus and potassium levels were compared. Plant growth was reduced in treatments containing low phosphorus levels when compared to the control. Nematode eggs and vermiform counts were reduced 25% and 57% in treatments with high levels of phosphorus, respectively. In the field study, sulfur and phosphorus were used as the treatments, because the soil was very low in those nutrients. The treatment effects were compared with a nematicide (1, 3-dichloropropene applied at 28.1 l/ha). Only treatments with the nematicide reduced reniform pathogenicity and reproduction. Sulfur and phosphorus treatments did not impact nematode reproduction at any of the three sampling times or result in enhanced yield. Both studies will be repeated this year for further investigation.

BIOCHAR INHIBITS HOST RECOGNITION BY *GLOBODERA TABACUM*. LaMondia, James A. The Connecticut Agricultural Experiment Station, Valley Laboratory, 153 Cook Hill Rd. Windsor CT 06095.

Biochar is an engineered charcoal soil amendment that sequesters carbon in soils and has been associated with increased plant growth and yield. Charcoal has high surface area and microporosity and is a strong adsorber of dissolved organic compounds. We investigated biochar for potential to reduce the bioavailability of host-specific hatch signaling compounds in soil. Cyst nematodes hatch in greater numbers in response to unknown signaling compounds in host-specific root exudates. We prepared root diffusates from tobacco or eastern black nightshade roots by soaking 8 g of root in 400 ml distilled water for 2 hrs. Diffusates were filtered and frozen until use. Full-strength or 1:10 and 1:100 dilutions of diffusates were percolated though 100 cm<sup>3</sup> pasteurized sandy loam soil or soil amended with biochar (Agrichar, Best Energies, Inc., Madison WI) at rates of 1% or 10% biochar by volume. Collected diffusates were then added to 5 or 6 replicate hatch chambers each containing 15 cysts of *Globodera tabacum* and the numbers of hatched juveniles counted over time. The experiment was conducted twice with similar results. Juvenile hatch from cysts exposed to diffusates leached through biochar-amended soil (P=0.002). Both 1% and 10% biochar amendments were effective in reducing juvenile hatch from full-strength root diffusate to levels similar to water alone or the 100-fold dilution of the root diffusate control, which were not different. Biochar may adsorb host-specific hatch signaling compounds, disrupting *G. tabacum* host recognition and subsequent hatch stimulation.

## ENTOMOPATHOGENIC SYMBIOSIS OF *CAENORHABDITIS BRIGGSAE* KT0001 AND *SERRATIA* SP. SCBI: ANALYSIS OF FITNESS. Lancaster, Jeremiah D., B. Mohammad, and E. Abebe. Department of Biology, Elizabeth City State University, 1704 Weeksville Road, Elizabeth City, NC, 27909.

Extensive research effort has advanced our understanding of *Caenorhabditis* as a model system, but its natural association with bacteria remains unexplored in an ecological context. Explored associations vary vastly from mutualistic to parasitic. *Serratia marcescens* has been shown to be pathogenic to *Caenorhabditis* with a fitness cost. The recent isolation of an entomopathogenic *Caenorhabditis briggsae* KT0001/S. *marcescens* SCBI association from the wild has allowed us to examine under laboratory conditions whether such an association poses a serious cost to *Caenorhabditis* as previously surmised for other *Serratia*. A fecundity table of *Caenorhabditis briggsae* KT0001 fed on *S. marcescens* SCBI and the control fed on *E. coli* OP50 is presented. We found no significant difference in survivorship or total fecundity between the *S. marcescens* SCBI fed and *E. coli* OP50 fed *Caenorhabditis briggsae* KT0001. Only the mean onset of reproduction was

significantly different between the two groups with *E. coli* fed *C. briggsae* maturing earlier (2.12 days) than those fed on *Serratia* (2.42 days). *S. marcescens SCBI* is most likely not pathogenic to *C. briggsae* KT0001 indicating that the entomopathogenicity reported for this association is beneficial for both the nematode and bacteria. In light of the fact that hitherto conducted experimental tests conform to widely held view that *Serratia* are pathogenic to *Caenorhabditis*, the absence of a fitness cost for *C. briggsae* we report here may indicate that this entomopathogenic association is non-transient–suggesting nematode/bacterial associations in the wild may vary greatly. Consequently, broad generalizations about nematode/bacterial associations should be interpreted with care.

EFFECTS OF ENVIRONMENTAL FACTORS ON THE SEX DIFFERENTIATION OF SOUTHERN ROOT-KNOT NEMTAODE (*MELOIDOGYNE INCOGNITA*). Lin, Yi-Hsin, Peichen Chen, and Tung-Tsuan Tsay. Department of Plant Pathology, National Chung Hsing University, 250 Kuo-Kuang Road Taichung 402, Taiwan.

The female root-knot nematodes have more deleterious effects on plants than males because they feed longer and can contribute to the secondary inoculum. The mitotically parthenogenetic *Meloidogyne incognita* juveniles have been reported to redirect development towards males when the host is under stress. In this study, five treatments were used to investigate their effects on the sex differentiation of *M. incognita*, including different pH treatment of juveniles, different N-P-K fertilizer proportions, pruning stress, methyl-jasmonic acid (MeJA) applications, and the host with resistant gene. When the pH 5 and pH 7-treated juveniles were inoculated on the water spinach in the pouch, they resulted in the most abundant males compared to other six pH treatments. However, in the pot tests, the pH 9 and pH 11-treated juveniles resulted in the most abundant males. When three N-P-K proportions were tested, the number of males from these treatments did not differ significantly. Plants treated with pruning stress yielded 339 males, representing 5.65% of the juveniles used as inoculum; while the unstressed plants yielded 22 males (0.37% of the inoculum). Application of 1.5 mM MeJA on plants resulted in the largest number of males (205 males, 3.42% of the inoculum); the 0.5 mM and 2.5 mM MeJA applications resulted in 71 (1.18%) and 17 (0.28%) males, respectively. The cowpea cultivar CB46 has a resistant *Rk* gene and CB46 NIL null is the near-isogenic line. The galling index on CB46 was relatively low, and the number of males was approximately seven times higher than that on the CB46 NIL null. Results showed that under alkalinity stimulations, pruning stress, 1.5 mM MeJA application and the presence of *Rk* resistant gene, the male differentiation proportion of *M. incognita* was significantly increased.

### A PRELIMINARY STUDY OF THE COMPOSITION OF THE GELATINOUS MATRIX OF *HETERODERA GLYCINES*. **Lopez-Nicora, Horacio D., and T.L. Niblack.** Department of Plant Pathology, Ohio State University, Columbus, OH 43210.

Heterodera glycines, the soybean cyst nematode, is a major pathogen of Glycine max (soybean). The H. glycines female lays some of her eggs into a gelatinous matrix (GM) which is not colonized by some of the most aggressive organisms used in biocontrol. GM is produced in the posterior part of the female, either by the vulvar or rectal glands. There is, however, limited information about the composition of the GM produced by H. glycines. Our hypothesis is that the GM contains chemicals or enzymes with antimicrobial activity. The objectives of this study were to confirm antimicrobial activity and to identify the components of the GM of H. glycines. A hydroponic system was used to produce virgin H. glycines females which produced egg-free gelatinous matrices. Females with GM were dislodged from roots with a high pressure water spray at 30 days post inoculation. Under a stereoscope at  $\times$  64 magnification ca. 200 females and GM were separated with forceps and the latter collected in 200 µL distilled water in 1.5 mL micro-centrifuge tubes. The samples were placed in a water bath at 70 °C for 24 hours to collect any diffusible compound in the aqueous phase. Gelatinous matrices, which are insoluble in water, were centrifuged and precipitated. The supernatant was removed and saved (fraction 1). To dissolve the GMs, 200 µL of 1N HCl was added to the tube (fraction 2). Protein content from both fractions was measured with a Bradford assay. Fraction 1 contained 42.5 µg/mL protein, whereas 1:10 dilution of fraction 2 contained 60.4 µg/mL protein. Both fractions were subjected to polyacrylamide gel electrophoresis under denaturing conditions (SDS-PAGE) and several peptide bands ranging in size from 10 kDa to >100kDa were observed. Metabolite profiling of both fractions with high performance liquid chromatography (HPLC) / mass spectrometry (MS) revealed several sugars, fatty acids and other metabolites of interest. Many of the metabolites have been reported to have or form part of compounds with antimicrobial activity, such as adipic acid, butylamine, hydroquinone, and lactic acid. Furthermore, when we plated GMs in nutrient agar with bacteria (e.g., Xanthomonas spp., Escherichia coli), inhibition of bacterial growth was observed as a clear area surrounding the GMs suggesting that the GMs exhibited antimicrobial activity. This may confer protection to the eggs against microorganisms, and may limit the potential for biocontrol organisms to manage H. glycines.

EFFICACY OF MULTIGUARD PROTECT® EC AT INCREASING SOIL DEPTHS IN TURFGRASS. Luc, John E. and William T. Crow. Entomology and Nematology Department, University of Florida, PO Box 110620 Natural Area, Dr., Gainesville, FL 32611-0620.

Multiguard Protect® EC, a commercial formulation of furfural, is a relatively new nematicide for turfgrass. Laboratory, greenhouse, and field trials in other crops have produced encouraging nematicidal results; however turf field trials have had

inconsistent results. Field trials were conducted to determine the effect of application method on the effectiveness of Multiguard Protect® EC at increasing depths of the soil profile in turf. Treatments consisted of: i)Multiguard Protect® EC 75L/ha with 0.64-cm of irrigation applied before and after application, ii) Multiguard Protect® EC 75L/ha followed by an additional 75L/ha applied after one hour with 0.64-cm of irrigation after each application, and untreated control receiving 1.28-cm of irrigation. Treatments were applied three times at two-week intervals to turf plots. The experimental design was a randomized complete block with 5 replications and conducted at two locations naturally infested with Belonolaimus *longicaudatus*. Nematode samples consisted of 8 cores (3.5-cm-diamter  $\times$  20-cm-depth) taken from each plot that were then cut into depth sections after removing the top 5-cm of turf and thatch. Soil depths observed were 0-5 cm, 5-10 cm, and 10-15 cm. Nematode and percent green cover data were collected to determine pretreatment levels, and then collected 2, 4, and 6 weeks after initial application. Root samples were collected pretreatment and 8 weeks later. Nematode reductions were observed between Multiguard Protect® EC and untreated control at several depths and dates during both trials. Multiguard Protect® EC 75L/ha + 75L/ha provided the most consistent nematode reductions. Furthermore, nematode reductions were most consistent at the two deepest soil depths. Differences in percent green cover were observed, but were inconsistent across trails. No differences between Multiguard Protect® EC and untreated control were observed during either trial for total root length. The lack of nematicidal effect within the top 5 cm of the soil profile may be due to chemical conversion, interaction with organic matter or chemical reaction within the aerobic fractions of the soil. Since nematode samples in turf are typically taken to a depth of 10 cm, the lack of nematicidal effect in the top 5 cm could mask or dilute the nematicidal effects occurring at deeper soil depths, resulting in the inconsistent results in earlier trials.

EFFECT OF MOVENTO ON COLUMBIA ROOT-KNOT NEMATODE AND POTATO YIELD. Luff, Kelly<sup>1</sup>, S.L. Hafez<sup>2</sup>, M.P. Pudasaini<sup>2</sup>, and R. Portenier<sup>2</sup>. <sup>1</sup>Bayer CropScience, 3554 East 4000 North, Kimberly, ID 83341; and <sup>2</sup>University of Idaho, Parma Research and Extension Center, 29603 U of I Lane, Parma, ID 83660.

An experiment was established at the University of Idaho, Parma Research and Extension Center to determine the efficacy of various treatments and combinations of Movento, Vydate and Vapam on Columbia root knot nematode. Ranger Russet potatoes were planted into a silt loam soil with an average initial nematode population of 352 per 500 cc soil. Treatments were replicated five times. Vapam was applied with a commercial fumigation bar set to a depth of 12-14 inches. Mocap was surface broadcast and incorporated 4 to 6 inches into the soil prior to planting. Vydate was placed in a 6-8 inch band in furrow at planting. Foliar applications of Vydate were applied with a small plot chemigation simulator and were repeated on a 14 day interval depending on the treatment. Movento treatments were initiated when sufficient foliage was present for uptake and were repeated on a 2 week interval depending on the treatment. MSO was combined with all Movento treatments. Results from this study show that Vapam alone or Movento in combination with Vydate or with Vapam + Vydate produced the greatest total yield. Clean tuber yield was highest in the Vapam + Movento or Vapam + Vydate + Movento treatments. The Vapam + Movento combination resulted in the lowest percentage of infected tubers (2.5%) and provided superior performance as compared to either product alone, Movento (38-42%) and Vapam (17%). Replacing three foliar applications of Vydate with two or three foliar applications of Movento resulted in nematode activity comparable to the Vydate program. In conclusion, fall application of Vapam followed by two foliar applications of Movento at 56 and 70 days after planting appears to be a promising treatment for the management of Columbia root-knot nematode in potatoes. Additionally, replacing foliar Vydate applications with Movento allows potato growers the opportunity to benefit from Movento's activity on aphids, psyllids, whiteflies, mites, thrips larvae and wireworm while maintaining nematode activity.

#### TRENDS IN NEMATODE PESTS DETECTED IN SOIL SAMPLES FROM COMMERCIAL CORN FIELDS. MacGuidwin, Ann<sup>1</sup>, and Bender, Breann<sup>1</sup>. <sup>1</sup>Plant Pathology Dept., University of Wisconsin, Madison, WI 53706.

There is a perception among lay audiences that the risk of corn yield loss due to nematodes has increased in the last 15 years. To test this hypothesis we examined data from 513 soil samples submitted to our lab for nematode assay. The samples were collected by agricultural professionals in June for corn surveys (1999, 2007, 2010) or from April 15<sup>th</sup> to June 30<sup>th</sup> (2000-2004, 2011) for diagnosis of corn problems. A 100 cm<sup>3</sup> subsample of soil was suspended in water and passed over a 250-µm-pore sieve nested over a 38-µm-pore sieve. Material retained on the top sieve was incubated on a Baermann Funnel for 48 hrs. Nematodes caught on the bottom sieve were separated from soil using sucrose centrifugation. Counts of nematodes from the two assays were summed for each sample to obtain the frequency of genera parasitic to corn in Wisconsin: *Pratylenchus*, *Hoplolaimus*, *Paratylenchus*, *Helicotylenchus*, *Tylenchorhynchus*, *Paratrichodorus*, *Mesocriconema*, *Xiphinema* and *Longidorus*. A nematode risk index was also computed for each sample by assigning a low- (10 points), moderate- (25 points), or high- (50 points) risk value for each genus present in the sample and summing the accrued points to one index score. The population density associated with each risk value was based on the consensus view of a nematology working group from seven Midwest states. Population densities per 100 cm<sup>3</sup> soil considered to constitute moderate risk for corn yield loss were 1 for *Longidorus*, 51 to 100 for *Hoplolaimus* and *Paratrichodorus*, 101 to 200 for *Pratylenchus* and *Xiphinema*, and over 500 for the remaining species. Only 2% of the samples were negative for any nematode pest of corn. *Pratylenchus* was the most prevalent genus detected (92%) and *Paratrichodorus* the least (3%). There were no temporal trends in the incidence of any genus. The

#### 476 Journal of Nematology, Volume 44, No. 4, December 2012

median population densities of *Pratylenchus* in 100 cm<sup>3</sup> soil ranged from 12 in 1999 to 116 in 2007 and showed a positive linear increase over time, as did population densities. The percentage of samples with population densities of *Pratylenchus* greater than 200 per 100 cm<sup>3</sup> soil increased from 3% to 28% from 1999 to 2007 and then declined to 12% and 15% in 2010 and 2011, respectively. The nematode risk index scores showed a small (.5 units year<sup>-1</sup>) but significant increase over the 12 year period, but the median score values were similar among years. Our results support the hypothesis that the risk of nematode damage to corn has increased in Wisconsin since the 1990's, particularly in regards to *Pratylenchus spp*. The bias inherent in diagnostic clinic samples warrant caution in applying these estimates of nematode severity to corn acreage at large.

*DITYLENCHUS WEISCHERI* AND NOT *D. DIPSACO* PRESENT ON CANADA THISTLE IN THE CANADIAN PRAIRIE PROVINCES. **Madani, M.<sup>1</sup>, M. Tenuta<sup>1</sup>, S. Briar<sup>1</sup>, and S.A. Subbotin<sup>2</sup>.** <sup>1</sup>Department of Soil Science, University of Manitoba, Winnipeg, MB, R3T 2N2, Canada; and <sup>2</sup>Plant Pest Diagnostic Center, California Department of Food and Agriculture, Sacramento, CA, 95832-1448.

The stem and bulb nematode (Ditylenchus dipsaci) was first described as a parasite of Canada thistle (Cirsium arvense) in the Canadian Prairie Provinces in 1979. Initially it was described as being Ditylenchus dipsaci. However, recently Ditylenchus on Canada thistle in central Russia was shown to be a new species, D. weischeri that is not considered an agricultural pest. This study examined if D. weischeri and not D. dipsaci is present on Canada thistle in the Canadian Prairie Provinces. Several Canada thistle plants were collected in fall 2011 from four fields of yellow pea (*Pisum sativum*) in each of the provinces of Manitoba, Saskatchewan and Alberta. All plants were infested with Ditylenchus except for samples from Alberta. Single to several individuals from each sample were used for molecular identification. The Internal Transcribed Spacer (ITS) region of rRNA gene including ITS1, 5.8S and ITS2 was amplified using the TW 81 and AB 28 primer set. For some samples the ITS1 and ITS2 were separately amplified using TW81 and Dit58SR or Dit58SF and AB28 primers, respectively. A fragment of heat shock protein gene (hsp90) was also obtained with U831 and L1110 primers. PCR-ITS products were subjected to restriction length polymorphism (RFLP) using five enzymes: Bsh1236I, HinfI, MspI, RsaI and TaqI. Comparative analysis of ITS and hsp90 gene sequences and PCR-ITS profiles of nematodes from our study match those published for D. weischeri. PCR with a species specific primer for D. weischeri was also designed and successfully tested using samples of Canada thistle containing the nematode and D. dipsaci from garlic (Allium sativum). The results of our study are of importance because Canada thistle seeds are common foreign material in marketed pea grain and calls to question if D. weisheri rather than D. dipsaci is the stem and bulb nematode occasionally found in export grain shipments.

#### NEMATODES ASSOCIATED WITH TURFGRASS OF KAUAI. Marahatta, Sharadchandra P., and P.V. Fewkes. Science and Math Division, Kauai Community College, 3-1901 Kaumualii Highway, Lihue, HI 96766.

A survey was conducted to determine the distribution and abundance of turfgrass-associated plant-parasitic (PPN) and free-living (FLN) nematodes on Kauai, Hawaii in Spring 2012. Turfgrass root and soil samples were collected from green and grounds of Waimea High School (Waimea), Kauai Community College (Puhi), and Kalena Park (Lihue). Root and soil nematodes were separately extracted using the Baermann funnel technique. PPN and fungivorous nematodes from roots, and PPN and FLN from soils were identified to genus level. Soil extracted nematodes were categorized as bacterivores, fungivores, PPN, omnivores, and predators. Nematode numbers under dominant trophic groups and FLN were separately compared. In root samples, Pratylenchus, Aphelenchoides, and Filenchus were found in all sampling sites. In Waimea, Ditylenchus dominated followed by Pratylenchus. Helicotylenchus was most common in Phui followed by Filenchus. Filenchus was most predominate followed by Meloidogyne in Lihue. The nematode richness associated with root samples in Waimea, Puhi, and Lihue were seven, six, and five, respectively. The nematode richness in soil samples was 17 in Waimea, 22 in Puhi, 33 in Lihue, and 34 altogether across survey sites. Only 15 nematode genera were consistently found in all sites. The most dominant soil extracted PPN were Pratylenchus followed by Helicotylenchus in Waimea, Helicotylenchus followed by Pratylenchus in Puhi, and Pratylenchus followed by Meloidogyne in Lihue. Less dominant PPN, Hoplolaimus, Mesocriconema, Paratichodorus, Paratylenchus, Radopholus, Rotylenchulus, and Xiphinema were found only in Lihue. Dominant FLN in Waimea, Puhi and Lihue were Eucephalobus followed by Acrobeloides, Filenchus followed by Aphelenchoides, and Filenchus followed by Eucephalobus, respectively. The dominance of bacterivorous nematodes indicated a nutritionally enriched soil in Waimea. However, comparatively unhealthy and stressed soil foodwebs were found in Puhi and Lihue as indicated by more herbivorous and fungivorous nematodes, respectively. The number of PPN genera and abundance of PPN could indicate damage from PPN in Kauai turfgrass. Therefore, a nematode management approach might be warranted in turfgrass.

DOES INTEGRATION OF HIGH AND LOW C:N RATIO COVER CROPS BENEFIT SOIL HEALTH MANAGE-MENT? **Marahatta, Sharadchandra P. <sup>1</sup>, K.-H. Wang<sup>2</sup>, and B. S. Sipes<sup>2</sup>.** <sup>1</sup>Science and Math Division, Kauai Community College, 3-1901 Kaumualii Highway, Lihue, HI 96766; and <sup>2</sup>Department of Plant and Environmental Protection Sciences, University of Hawaii, 3050 Maile Way, Honolulu, HI 96822.

Organic matter with high and low C: N ratios stimulate fungal and bacterial decompositions, respectively. The objective of this study was to evaluate if combination of high and low C: N ratio materials enhance both decomposition channels, and thus

further improve soil health. Three field trials were conducted using nematodes as soil health indicators. Sunn hemp (SH) (Crotolaria juncea) and oat (O) (Avena sativus) were used as low and high C: N cover crops, respectively. All cover crop treatments were in a strip-till living mulch system. SH living mulch was periodically clipped and left on the soil surface as surface mulch. In Trial I, nematode communities in kabocha squash (Cucurbita maxima) grown in SH were compared to bare ground (BG). At termination of squash crop, numbers of fungivorous and predatory nematodes were higher in SH than BG (P < 0.10). Trial II and III were superimposed on treatment plots in Trial I with two additional treatments, i.e. SH, O, SH+O and BG. At the end of Trial II, SH+O increased (P < 0.05) numbers of bacterivorous and fungivorous nematodes, O increased (P < 0.05) fungivorous as compared to SH and BG, both SH or SH+O reduced herbivorous nematodes (P > 0.10), and SH increased (P < 0.05) omnivorous nematodes. Effect of cover crops on predatory nematodes tend to be higher in SH or SH+O at squash harvest, but not significant. Only SH+O reduced channel index (P < 0.05), an indication of reduced stress of soil health. In Trial III, cantaloupe (Cucumis melo var. cantalupensis) was interplanted into SH, O, SH+O and BG plots. At cantaloupe harvest, all cover crops increased bacterivorous nematodes (P < 0.05), SH+O was the only treatment that suppressed reniform nematodes (Rotylenchulus reniformis), whereas O resulted in higher root-knot nematodes (combination of *M. javanica* and *M. incognita*) than BG. SH or SH+O increased (P < 0.05) enrichment index at cover crop incorporation, and reduced (P < 0.05) channel index at cantaloupe harvest, indicating enhancement of nutrient enrichment and reduced stressful condition. Estimation of C: N ratio of SH leaf, flower, stem and whole plant of O at  $\sim$  3 months after planting were 8.86, 10.14, 39.28, and 27.19: 1, respectively. Overall, SH+O tended to outperform O, but had similar effects as SH with occasional performance better than SH in terms of enhancing free-living nematodes. Better performance of SH+O than SH could be due to a flour beetle affecting the growth of sunn hemp in SH plots but not SH+O plots due to shorter planting history of SH+O. In conclusion, adding O to SH did not improve soil health condition more than SH alone. This is attributed to the diverse C: N ratios of different SH tissues.

VARIATION IN ECLOSION AND HATCH OF EGGS AMONG GEOGRAPHIC ISOLATES OF *ROTYLENCHULUS RENIFORMIS*. McGawley, E.C.<sup>1</sup>, C. Overstreet<sup>1</sup> and M.J. Pontif<sup>2</sup>. <sup>1</sup>Louisiana State University AgCenter, Department of Plant Pathology and Crop Physiology; and <sup>2</sup>Sugarcane Research Station, Baton Rouge, LA 70803, USA.

Studies published previously have documented the role of weed exudates on the eclosion and hatch of eggs of *Roty-lenchulus reniformis* and differences in eggs per gram of root tissue produced by pathologically variable geographic isolates of *R. reniformis* from LA, MS, TX, HI and AR. Laboratory studies conducted subsequent to microplot-based pathogenicity experiments evaluated more precisely the eclosion and emergence of juveniles of *R. reniformis* from eggs representative of the 5 geographic isolates. For each isolate, 6 replicates of 50 hand-picked, undifferentiated, freshly-extracted eggs were placed into 20 ml of sterile distilled water (pH 7.1-7.3) contained in a 60 x 15mm Petri dish and incubated at 28C. Contents of each dish were examined daily for 13 days and categorized into six developmental stages: undifferentiated egg; 8-16 cell stage egg; juvenile inside egg; hatched juvenile; infective female and male. The study was repeated twice and data analyzed over the 3 runs of the trial. At 96h, some eggs of all populations had reached the 8-16 cell stage, although development was significantly less so for the populations from HI and AR. By day 7, hatched juveniles and/or juveniles in eggs from all populations except those from HI were hatched. At 13 days, populations from LA and MS contained only juveniles, infective females and males. Those from TX, HI and AR remained 10-25% mixed egg stages, most of which did not hatch by 20 days when monitoring was terminated. These differences in egg and juvenile development among populations correlate well with final population densities and subsequent pathogenicity data from full-season microplot trials with cotton and soybean.

### NEMATODE CONTROL FOR GRAPEVINES IN THE NURSERY, AT PLANTING AND FOR DECADES BEYOND. **McKenry, Michael.** University of California, Dept of Nematology, Riverside, CA 92521.

Tools for producing nematode-free nursery stock have been available to California growers since the early 1960s. The expensive three to four step process assuring plant sanitation continues into this century as greater attention has been focused on soil moisture content in order to reduce treatment rates while maintaining fumigant availability. As an update, incidence of nematode invasion is four times as likely following non regulated nursery crops eg. turf, compared to regulated nursery crops specifically intended for California farmlands. Google NIPM #7 for the most current tactics which include soil fumigation requirements, non host periods, root inspection and for grapes may also include soil solarization of liner plants or hot water treatment of rootings. As clean plants are moved into previous vineyard sites their average first year vigor in non-fumigated soil may average 1/7<sup>th</sup> of that in fumigated soil. Root rejection can be a devastating component of the replant problem (RP). Within two years after planting the root rejection component is gone but the soil pest component of RP remains for the lifetime of the perennial. Nematode resistance mechanisms within grapevines can persist for decades if they occur broadly across the root system and in every locale each different nematode species may desire to feed. Recently available is the first grape rootstock (Demko10-17A) having tolerance to root rejection while also providing broad and durable resistance to major nematode pathogens of grape. Grapes currently provide the best example of successful 'Starve' & 'Switch', a new alternative to soil fumigation. Specifically for grapes we saw off old vine trunks in February-March, paint glyphosate

#### 478 Journal of Nematology, Volume 44, No. 4, December 2012

herbicide onto the remaining cut trunk, wait a full year and then switch to a rootstock having very different parentage. The root starvation effort is important but the switch to a root of different parentage is very important. For most perennial crops we now have the new post-plant nematicide spirotetramat available as an alternative to organophosphate and carbamate nematicides. Safety and environmental features associated with this foliar-applied nematicide greatly exceed those of its predecessors while providing nematode control at least as useful and accomplishing this without disrupting natural antagonists such as *Pasteuria* spp. The breadth of pest relief provided by Demko10-17A and Spirotetramat also deters other root feeders such as grape phylloxera and the root inhabiting stage of grapevine mealybug.

#### AN OVERVIEW OF ORGANIC AMENDMENT USE FOR MANAGEMENT OF NEMATODES ON FLORIDA VEG-ETABLE CROPS. **McSorley, Robert.** Dept. of Entomology and Nematology, University of Florida, PO Box 110620, Gainesville, FL 32611-0620.

Organic amendments have been widely used for nematode management, although results have been variable. Effects of organic amendments on plant-parasitic and free-living nematodes were reviewed from a variety of tests conducted on sandy soils in Florida. Yard waste composts (YMC) with high C:N ratios (>30:1) rarely affected numbers of plant-parasitic nematodes, but increased crop yields in 9 of 20 field trials. In another series of studies, fresh crop residues or composted municipal solid wastes (MSW) reduced numbers of root-knot nematodes in 45% of tests with these materials, and increased crop yields in 67% of cases. When amendments were compared directly with methyl bromide (MB), the fumigant was far superior in suppressing root-knot nematodes in 6 tests. In two trials in which MB and amendments gave similar results, the amendment treatment consisted of a biosolarization with MSW under clear plastic. In 6 tests that examined impact of amendments on free-living nematodes, all materials used (crop residue, YWC, MSW, chicken manure) increased numbers of bacterivorous nematodes and most stimulated fungivores as well, while effects on omnivores varied with the materials used. Overall, most amendments were effective in stimulating free-living nematodes, particularly bacterivores, and many amendments improved plant yield. However, effects on plant-parasitic nematodes varied from rare with YWC to inconsistent with crop residues or MSW. Although beneficial effects were noted, amendments do not appear to be a consistent and reliable method for managing plant-parasitic nematodes under field conditions in Florida. Consistency might be improved by optimizing or combining some of the more promising materials and techniques.

EFFECTS OF AGRONOMIC PRACTICES ON THE ESTABLISHMENT OF *HETERODERA GLYCINES* IN VIRGIN LAND. **Melakeberhan, Haddish<sup>1</sup>, A. Kravchenko<sup>2</sup>, and K. Thelen<sup>2</sup>.** <sup>1</sup>Agricultural Nematology Lab, Department of Horticulture; and <sup>2</sup>Department of Crop and Soil Science, Plant and Soil Science Building, Michigan State University, East Lansing, MI 48824.

A lot is known about the soybean cyst nematode's (SCN, *Heterodera glycines* Ichinohe) increasing distribution, presence of parasitic (genetic) variability, and its economic significance in a wide range of soybean production landscapes. However, little is known about how SCN adapts and increases to damaging levels when introduced into SCN-virgin land and subjected to agronomic practices. In 2001, SCN, race 3 (Hg Type 0) was introduced into 20 x 10 ft plots under till and no-till, and either corn (C), SCN- resistant soybean (R), or SCN-susceptible soybean (S) monocrop, or RCRC and SCSC rotation cycles and augmented to approximately 400 eggs/100 cm<sup>3</sup> of soil in 2002. Treatments were replicated four times and equal numbers of non-infested plots served as controls, for a total of 80 experimental plots. Soil texture across tillage and nematode treatments was  $60 \pm 1\%$  sand,  $13 \pm 1\%$  silt, and  $26 \pm 1\%$  clay. Over the course of six years (2003 – 2008), SCN population density, plant stand, and yield were measured. Few cysts were detected in non-infested plots. In 2005 and 2004, the population density remained less than 1 cyst/100 cm<sup>3</sup> of soil and reached the maximum to less than 10 cysts/100 cm<sup>3</sup> in 2005 and 2006. In all cases, the population density was highest in S and lowest in C or RC rotations. In 2007 and in 2008 stand count was less in tilled than in no-till plots. Soybean yield was similar between nematode treatments until 2006. In 2007, in both tillage systems, and in 2008, in no-till plots, yield of SCN-infested plots was significantly lower than non-infested plots. The study provides agro-biologically based timeline information that is critical for SCN management.

### A TECHNIQUE TO EVALUATE THE EFFICACY OF CHEMICAL COMPOUNDS ON PLANT NEMATODES. **Mendes, Maria de Lourdes, and D.W. Dickson.** University of Florida, Entomology and Nematology Department, P.O. Box 110620 Gainesville, FL 32611-0620.

A technique using PVC pipe was developed to evaluate the effectiveness of experimental compounds on root-knot nematode development under laboratory conditions. This apparatus consists of two pieces of PCV pipe each 2.5 cm in diameter (diameter can vary). The top piece, 5 cm long, forms the plant containment chamber. A Nitex cloth with 38 µm diameter openings is glued tightly to the bottom of this chamber. This cloth prevents roots from growing into the second chamber, but does not stop nematodes from migrating into the containment chamber. The bottom piece, 15 cm long forms the nematode mobility and treatment chamber. Both pieces are filled tightly with pasteurized masonry sand. A single 1-week old root-knot nematode susceptible tomato seedling is transplanted into the root containment chamber and incubated in a precision incubator at 28 C. A fertilizer solution containing 0.21g/L of water of 20-20-20 NPK was applied every 2 days. A

week later, after the root system became established treatments are applied to the treatment chamber. The chemical solutions prepared with water are drenched over the top of the mobility and treatment chamber in 5 mL of the appropriate dilutions and set aside to allow the material to flow thorough the sand medium to reach the bottom of the tube. The control is treated with 5 mL of water. Twenty-four hours after the treatment application, the tubes are turned upside down and 300 second-stage juveniles of *Meloidogyne* spp. are added in 2 mL of water. The chambers are set aside for at least 4 hours to allow the nematode suspension to flow into the sand. After that, the plant chamber is nested over the mobility and treatment chamber and taped together. There has to be complete contact between the two chambers. The set is placed in a 150 mL beaker and incubated at 28 C. The plants are watered every other day (or when needed) by adding 2 mL of water to the beaker. At 13 to 15 days after inoculation the root chamber is removed, and the root system washed clean. The roots are cleared, stained, and the juveniles that penetrate them are counted under an inverted microscope. This method provides detailed information on effectiveness of treatments on root-knot nematode development.

COMPARATIVE ANALYSES OF NEMATODE COMMUNITY AND ECOSYSTEM SERVICES IN AGRICULTURAL AND NATURAL ECOSYSTEMS OF SELECTED MICHIGAN SOIL GROUPS. Mennan, Sevilhan<sup>1,6</sup>, J. van Ravansway<sup>2</sup>, Z. Cheng<sup>3</sup>, H.K. Bal<sup>3</sup>, P.S. Grewal<sup>3</sup>, A.J.M. Smucker<sup>4</sup>, A. Adelaja<sup>5</sup>, J. Warbach<sup>5</sup>, J. Qi<sup>2</sup>, and H. Melakeberhan<sup>1</sup>. <sup>1</sup>Agricultural Nematology Laboratory, Department of Horticulture; <sup>2</sup>Center for Global Change and Earth Observations; <sup>4</sup>Department of Crop and Soil Science; and <sup>5</sup>Land Policy Institute, Michigan State University, East Lansing, MI 48824; <sup>3</sup>Department of Entomology, The Ohio State University, OARDC, Wooster, OH 44691; and <sup>6</sup>Ondokuz Mayis University, 55139 Samsun, Turkey.

Michigan is among the states with glacial soil deposits that fall in a category of multiple levels of degradations, and is dominated by the northward infusion of intensive production of multi-purpose crops such as corn and soybean. The result is often conflicting agronomic, ecological, economic, and biological outcomes associated with changing land use. However, improvements can be made through an integrated understanding of soil ecosystem services for which nematode assemblage analyses is a key indicator. The overall project goal is to establish relationships between nematode assemblage and soil groups (orders), ecosystem degradation, and soil nutrient cycling potential in distinct temperature zones within the lower peninsula of Michigan. We investigated natural (pristine forests and native succession vegetation) along with disturbed landscapes associated with agricultural soils having altered biological functions and soil nutrients on selected Udalfs, Psamments and Saprists soil sub-orders within northeast (NE) and southwest (SW) temperature zones of 40.1 - 45.0 °F and 45.1 - 50.0 °F, respectively. The NE and SW locations were about 300 miles apart between the latitudes of 42° and 45°. The disturbed and natural landscapes within the soil groups were identified using Google Earth and digitized state soil maps. Within a landscape, two to three fields were selected, and 5-10 geo-referenced samples per field collected from 0-30 cm and 30-60 cm depths. Soil properties, nematode assemblage, and bio-control activities were analyzed by soil group. Temperature, landscape and sampling depth and appropriate interactions were also tested. Soil moisture, soil organic matter (SOM), and natural bio-control activity in the top 30 cm revealed no difference between NE and SW locations. However, soil moisture and SOM were higher in disturbed areas than in natural areas. No entomopathogenic nematodes were detected in any of these locations, but total and entomopathogenic fungi-based potential bio-control activities were higher in natural areas than in disturbed areas. Bacteriovore and fungivore nematodes appear to vary by soil group; whereas, herbivore and predacious nematode groups varied by temperature and soil groups. Overall, there were significant two-way and/or three-way interaction effects of the independent variables on nematode assemblage parameters, suggesting that the same soil groups may have different biological structures and/or functions within the different temperatures zones and disturbance regimes.

MIXED SPECIES COVER CROP GREEN MANURES FOR MANAGEMENT OF SOILBORNE PATHOGENS ON TOMATO. **Meyer, Susan L.F.<sup>1</sup>, K.L. Everts<sup>2</sup>, and B.B. McSpadden Gardener<sup>3</sup>.** <sup>1</sup>USDA-ARS Nematology Laboratory, Beltsville, MD 20705; <sup>2</sup>University of Maryland, Salisbury, MD and University of Delaware, Georgetown, DE; and <sup>3</sup>Ohio State University, Wooster, OH.

Rising organic vegetable production and sales have generated an increased need for pest management strategies compatible with organic cultural practices. Winter cover crops, which are commonly used in organic farming, were incorporated as green manures in the spring and effects on nematodes, soilborne pathogens and tomato fruit yields were evaluated. Studies conducted in 2010 and 2011 were designed to compare mixed species green manures and single species manures for improving soil and plant health. The treatments were 1) mixed species hay (*Festuca arundinacea*, tall fescue; *Dactylis glomerata*, orchard grass; *Phleum pratense*, timothy; *Trifolium pretense*, red clover; and *Medicago sativa*, alfalfa); 2) *Vicia villosa* (hairy vetch); 3) *V. villosa* and *Secale cereale* (rye); 4) *V. villosa* and *Raphanus sativus* (forage radish); and 5) a bare ground control. There were six plots per treatment each year, and each was sampled four times for plant-parasitic nematodes: 1) prior to green manure incorporation, 2) ca. 2 weeks later, 3) midseason and 4) at harvest. Southern blight and early blight were evaluated during the growing season. Total populations of plant-parasitic nematodes were low (overall means 25.5 and 11.1/100 cc soil per plot in 2010 and 2011, respectively). Overall, *Tylenchorhynchus* spp. were the most frequently counted from the plots each year. They occurred in ca. 30-47% and 6-17% of the 30 plots in 2010 and 2011, respectively, varying with sampling date. Nematode pressure was therefore low. No significant differences (P < 0.05) in numbers of PPN were found among sampling dates or treatments. Early blight was greater (P = 0.0012) in tomatoes grown in plots with a hairy vetch green manure than in all other treatments, while southern blight was greatest (P = 0.0100) in hairy vetch plus rye plots. In 2011, disease severity was low and there were no differences among green manure treatments. When disease pressure was moderate, mixed species hay (Treatment 1) and hairy vetch plus forage radish (Treatment 4) resulted in improved disease suppression compared to hairy vetch (Treatment 2) or hairy vetch plus rye (Treatment 3).

#### ESTABLISHING STANDARDIZED METHODS FOR ANALYSIS OF CARBON IN SOIL NEMATODES AND MITES. Milano de Tomasel, Cecilia, K.L. Ivanovich, D.J. Cox, E.A. Shaw, and D.H. Wall. Department of Biology and Natural Resource Ecology Laboratory, Colorado State University Fort Collins CO 80523.

Soil biotic interactions are essential to belowground processes, such as soil carbon (C) dynamics. Microfauna is known to be a major contributor to soil C cycling, however there is a need for more information about the role of individual groups in soil-C dynamics. Thus, accurate measurement of microfauna-C is essential to understanding how groups like nematodes and mites contribute to the soil C cycle. Stable isotope analysis is a common technique for measuring elemental dynamics of ecological processes, and could be a useful tool for quantifying the flow of C through nematodes and mites. Although many studies have been dedicated to understanding stable isotope dynamics of <sup>13</sup>C in the soil and atmosphere, the methods for quantifying <sup>13</sup>C content of and flow through soil animals has not previously been tested or standardized. In this experiment, we determined the minimum number of specimens necessary to detect and quantify a consistent amount of <sup>13</sup>C in nematodes and mites. Nematodes were extracted by the Baermann funnel method from laboratory samples containing Plectus murrayi. The experimental design for nematodes consisted of three replicates of eight treatments: 35, 45, 55, 65, 75, 100, 150, and 200 nematodes. Mites were extracted by Tullgren funnel method from soil samples collected at Konza Tallgrass Prairie Long Term Ecological Research site. The experimental design for mites consisted of four replicates of four treatments: 1, 5, 10, and 20 mites. Both nematodes and mites were handpicked into tin cups under a dissecting microscope (60X) and sent to the Stable Isotope Mass Spectrometry Laboratory at Kansas State University for elemental analysis. Results indicated that at least 75 nematodes and 20 mites are necessary to obtain low variability <sup>13</sup>C measurements. The results of this study will inform future studies involving soil microfauna-C measurement, such as food web studies of C flow dynamics.

#### LIFE CYCLE OF *GLOBODERA ROSTOCHIENSIS* IN QUEBEC, CANADA. Mimee, Benjamin, and G. Bélair. Agriculture and Agri-Food Canada, St-Jean-sur-Richelieu, Quebec, Canada J3B 3E6.

In 2006, the golden nematode (*Globodera rostochiensis*) was discovered in the province of Quebec, Canada. Recent molecular analyses have demonstrated that this nematode was not genetically related to other North American populations suggesting that this new outbreak could have been caused by a second introduction of potato cyst nematode, most likely from Europe. The recording of the life cycle of this Canadian population has revealed that only one generation was completed per year in the climatic conditions of Quebec. First mature cysts appeared 50-56 days after planting, which are annually synchronized with the apparition of the first flower buds on the potato plant. In soil, the first second-stage juveniles were recorded 14 days after planting while both white females on roots and males in soil appeared synchronously after 35 days. A second wave of hatching systematically occurred later in the season, at flowering. Even if they invaded the plant, no females were observed on the roots and no males were found in soil at this time. In 2011, this second peak of hatching was particularly abundant where the number of J2 larvae was increased by 2-3 times when compared to the first peak. Thus, immature cysts were able to hatch in response to root exudate before entering the dormancy phase. In addition, two distinct patterns of hatching and cysts development, staggered over a 3-week period, were observed simultaneously in several microplots and suggest the presence of two subpopulations.

## SYSTEMIC ACTIVITY OF FLUENSULFONE FOR CONTROL OF *MELOIDOGYNE INCOGNITA* ON VARIOUS VEGETABLE CROPS. Morris, Kelly<sup>1</sup>, D.B. Langston<sup>1</sup>, J.P. Noe<sup>2</sup>, and W.T. Holladay<sup>2</sup>. <sup>1</sup>Department of Plant Pathology, University of Georgia, Tifton, Ga. 31793; and <sup>2</sup>Department of Plant Pathology, University of Georgia, Athens, GA 30602.

Fluensulfone is a new nematicide that is a member of the flouroalkenyl chemical group. The systemic activity and phytotoxicity of this compound was tested during the spring of 2012 on four different vegetable crops: tomato (Florida 47), eggplant (Night Shadow), cucumber (Rockingham), and squash (Payroll). Seedlings of each crop were planted into 7cmx25cm black conetainers containing a potting mix of approximately 90% sand and incubated in a growth chamber at 28°C with 12h photoperiod. Conetainers were arranged in a randomized complete block design with six replications per treatment. Nematicide treatments were applied two days after transplant and conetainers were inoculated with 1,500 *Meloidogyne incognita* Race 1 J2 two days after nematicide treatment. Treatments consisted of fluensulfone at rates of 3, 6, and 12 g a.i/l, oxamyl at a rate of 4.8 g a.i./l, an inoculated nontreated replication, and a noninoculated nontreated replication. Treatments were applied via backpack sprayer calibrated to deliver 238 liters/ha using T-Jet 8004 tips. Prior to being treated, the base of the plant was secured tightly with plastic wrap and rubber bands to prevent nematicide contact with the soil. Plant heights were measured and a vigor rating was recorded 12 d after treatment to assess phytotoxicity. Four weeks after

inoculation fresh weights of plant tops and roots were recorded and galls and nematodes were counted after roots were stained with acid fuchsin. Reported differences are significant at  $P \le 0.05$  with the exception of cucumber. Because of problems with *Pythium* contamination in cucumber pots these differences are shown at  $P \le 0.10$ . The high rate of fluensulfone reduced nematode counts and galling in tomato roots by 58% and 46%, respectively, compared to the nontreated check. Oxamyl significantly reduced nematode counts in roots and galling on tomato by 74% and 73%, respectively, compared to the nontreated check. Neither nematicide demonstrated systemic suppression of nematode counts in roots or galling in the other crop species tested. The high rate of fluensulfone reduced plant vigor and height in eggplant by 68% and 33% respectively. A 15% reduction in vigor of tomato was observed for rates of 3 and 6 g a.i./l and 33% reduction in vigor at the 12 g a.i./l rate, however, root weights for the low rates of fluensulfone were higher than the root weights of the nontreated tomato plants. The high rate of fluensulfone reduced vigor in cucumber by 28%. Phytotoxicity was not observed in squash at any rate. Similarly, phytotoxicity was not observed in any crop for the oxamyl treatment. Fluensulfone has systemic activity when applied as a foliar spray in certain crops, such as tomato, but also can be phytotoxic to other crops, such as eggplant. Oxamyl only showed systemic activity in tomato in these experiments. Similarly to fluensulfone, systemic activity of oxamyl appears to be crop-dependent.

SOIL NEMATODE GENERA THAT PREDICT SPECIFIC TYPES OF DISTURBANCE. Neher, Deborah A.<sup>1</sup>, and Zhao, J.<sup>1,2</sup>. <sup>1</sup>Department of Plant & Soil Science, University of Vermont, 63 Carrigan Drive, Burlington, VT 05405; and <sup>2</sup>Key Laboratory of Vegetation Restoration and Management of Degraded Ecosystems, South China Botanical Garden, Chinese Academy of Sciences, Guangzhou 510650, PR China.

Nematode community indices would be more cost-effective and interpretable if ambiguous genera were removed and indices reduced to include genera with known sensitivity or response to specific types of disturbance. The objective of the present study was to perform a meta-analysis of existing datasets of high quality and enumerate the genera that response universally consistency to specific disturbance, treatment, or management worldwide. We collected 21 sources of original data primarily from farmland and secondarily from grassland, forest and orchard with manipulated treatments in cultivation, inorganic or organic fertilization and identified whether samples were collected in the spring, summer or autumn. Canonical correspondence analysis was used to determine the effect of disturbance type on the composition of soil nematode community composition. Genera that performed consistently in a single direction and across at least two seasons were identified. Briefly, cultivation reduced abundances of *Diphtherophora*, *Prismatolaimus* and *Tylenchorhynchus*. Application of synthetic chemical fertilizers reduced numbers of *Plectus*. Application of organic fertilizers resulted in increased numbers of *Cruznema*, *Mesorhabditus*, *Mesodorylaimus* and *Nygolaimus*. No genera met the criteria for responding positively to either tillage or inorganic fertilization or negatively to organic fertilization. The source of nutrients apparently affected nematode communities differently. These genera need to be verified by independent data to confirm that they generally reflect intensive cultivation or fertilization by synthetic or organic types. Once verified, this subset of genera will improve interpretation of index values and can be the initial targets for developing molecular probes that can be made accessible to non-specialists.

#### INTERACTIONS AMONG ENTOMOPATHOGENIC NEMATODES AND OTHER NEMATODE TROPHIC GROUPS AND PLANTS IN AGROECOSYSTEMS. **Nethi, Somasekhar<sup>1</sup>, G.B. Jagdale<sup>2</sup> and P.S. Grewal<sup>3</sup>.** <sup>1</sup>Directorate of Rice Research, Rajendranagar, Hyderabad, 500030, India; <sup>2</sup>Plant Pathology Dept., University of Georgia, Athens, GA 30602; and <sup>3</sup>Department of Entomology, Ohio State University, OARDC, Wooster, OH 44691.

Entomopathogenic nematodes together with their symbiotic bacteria represent an important biological control system. These nematodes are being increasingly used for the control of soil pests in many crops worldwide due to their positive attributes and exemption from registration requirements in many countries. Available information from field studies suggests that predictability of control, an important consideration in biological control programs can only be achieved with entomopathogenic nematodes by deploying right nematode species/isolate against right pest in the right ecosystem. This essentially requires precise knowledge of evolutionary relationships and multitrophic interactions of entomopathogenic nematodes in soil food webs. In this context, we have addressed interactions of entomopathogenic nematodes with other nematode trophic groups and plants in agroecosystems. Although, inundative field applications of entomopathogenic nematodes were shown to have no significant long-term adverse impact on non-target arthropods, improvements in plant growth were observed in fields treated with entomopathogenic nematodes in some areas. This plant growth improvement was attributed to the decrease in abundance of plant parasitic nematodes following application of entomopathogenic nematodes. Following this, there was a surge in reports on suppression of plant parasitic nematodes by entomopathogenic nematodes. The interaction between entomopathogenic nematodes and plant-parasitic nematodes has become a subject of intense study from both ecological and commercial perspective over the past two decades. While suppression of plant parasitic nematodes, though a non-target effect, is considered beneficial from the pest management perspective, concerns were raised about its mechanisms and the possible adverse impact of entomopathogenic nematodes on other nematode trophic groups in soil and ecosystem services they provide. Our efforts to address these key issues resulted in unravelling of a unique phenomenon of selective suppression of plant parasitic nematodes by entomopathogenic nematodes without any adverse impact on beneficial free-living trophic groups (bacterivores, fungivores, predators, omnivores) of nematodes in soil food webs. This effect was referred as a beneficial non-target effect of entomopathogenic nematodes. These findings gave further impetus to the studies on the mechanisms underlying suppression of plant parasitic nematodes by entomopathogenic nematodes. Recent studies have demonstrated that the entomopathogenic nematodes and their symbiotic bacteria can induce systemic resistance in plants which may act against plant parasitic nematodes. This gives an insight into how entomopathogenic nematodes could selectively suppress plant parasitic nematodes in soil ecosystem. Our current understanding of the interaction of entomopathogenic nematodes with other trophic groups of soil nematodes and plants, its ecological significance and consequences for their successful use in biological control programs are discussed in the light of recent developments in the field of entomopathogenic nematology.

ECOLOGY OF SOILS SUPPRESSIVE TO SOYBEAN CYST NEMATODE: III. ASSOCIATION OF NEMATODE AND MICROBIAL COMMUNITIES WITH SOIL SUPPRESSIVENESS. Nishanthan, Tharshani<sup>1</sup>, Deborah A. Neher<sup>1</sup>, and Senyu Chen<sup>2</sup>. <sup>1</sup>Department of Plant & Soil Science, 63 Carrigan Drive, Burlington, VT 05405; and <sup>2</sup>University of Minnesota Southern Research and Outreach Center, 120th Street, Waseca, MN 56093, USA.

The long-term goal of this project is to develop ecologically-based, sustainable management of the soybean cyst nematode by characterizing the composition and function of suppressive soils and by understanding how production practices affect biological suppression of the soybean cyst nematode. The general working hypothesis is that certain production practices will alter soil community composition and function that create long-term suppression of soybean cyst nematode (Heterodera glycines, SCN) populations and/or manifestation of disease. A field experiment was designed as a split plot and replicated four times in two fields naturally suppressive to SCN in Waseca County, Minnesota. Main plots were cultivation (no till, conventional till) and subplots were five crop-biocide combinations. Treatments were chosen to identify management practices that disrupt natural suppression of SCN. Soil samples were collected three times per year (planting, mid-season, harvesting). Nematodes were enumerated and identified to genus. Activity of fourteen extracellular enzymes was quantified to assess function of the decomposer microbial community. Cultivation, application of biocides, and rotation to corn all reduced suppression of SCN and the impact increased progressively within the first three years of the four year experiment. There was a significant two-way interaction between cultivation and crop-biocide treatments. Abundance of plant-parasitic and fungivorous nematodes decreased and abundance of bacterivorous nematodes increased with cultivation. Among plantparasitic nematodes, the proportion that was *Helicotylenchus* was correlated negatively with *Heterodera glycines*. When soybean was rotated to corn, the relative abundance of fungivorous nematodes (especially Aphelenchoides) increased. Naturally suppressive soils contained greater activity of chitinase than conducive soils. Preliminary pyro-sequencing analysis of bacterial communities suggests Verrucomicrobia were more abundant and Actinobacteria were sparse in suppressive soils. Values of  $\Sigma$ MI25 and trophic diversity indices were correlated positively with SCN suppressive soils. Based on these results, there appears to be some association between *Heterodera glycines* and *Helicotylenchus* in the rhizosphere, perhaps competing for space and/or nutrients. Mannase and arabinase seem to be related to crop rotation, reflecting different proportions of carbohydrate monomers in the cell wall of corn than soybean. Our results suggest that disease suppression appears to be more closely aligned with fungi than bacteria. Natural suppression of SCN appears to be associated with the microbial community fostered by a combination of no-till and soybean monoculture.

## PREPLANT SOIL TREATMENTS TO MANAGE BLUEBERRY REPLANT DISEASE CAUSED BY MESOCRICONEMA ORNATUM. Noe, J.P., P.M. Brannen, W.T. Holladay, and G.B. Jagdale. Plant Pathology Dept., University of Georgia, Athens, GA 30602.

Blueberry production in Georgia has a farm gate value in excess of \$100 million and accounts for almost one-third of the total fruit and nut crop value for the state. A slow decline in plant vigor and an associated replant disease has been observed on a number of blueberry farms, which led to an investigation of possible causes. Soil assays showed that Mesocriconema ornatum was associated with the plant growth symptoms. After confirmation of pathogenicity in greenhouse and microplot experiments, a field study was undertaken to determine the efficacy of preplant soil treatments to manage M. ornatum on blueberry. Experimental plots were established on two blueberry farms located in two Georgia counties, Appling and Bacon, that were previously planted in blueberry and were naturally infested with M. ornatum. Treatments included preplant fumigation with broadcast equivalent rates of methyl bromide/chloropicrin (50:50) at 448 kg/ha, 1,3-dichloropropene at 91 and 273 liter/ha, preplant solarization of the soil under clear plastic for 77 days, and nontreated controls; 6 replicates of each treatment were administered at each site. Data were combined from both sites for analysis. Soil assays conducted immediately after plastic film was removed showed that soil treatment with solarization reduced population densities of *M. ornatum* by 64% compared with nontreated plots ( $P \le 0.05$ ). Preplant soil treatment with methyl bromide/chloropicrin, or 1,3-dichloropropene at 91 or 273 liter/ha reduced population densities to 3, 6, and 0 M. ornatum/ 100 cm<sup>3</sup> soil, respectively, compared with 203 M. ornatum/ 100 cm<sup>3</sup> soil in the nontreated plots ( $P \le 0.05$ ). In soil assays taken after fumigant treatments were applied and planting beds were formed, population densities in the solarized plots remained lower (140 M. ornatum/ 100 cm<sup>3</sup> soil) than the nontreated plots but were higher than all the soil fumigant treatments (P $\leq$ 0.05). After planting,

population densities of *M. ornatum* and other plant-parasitic nematodes and blueberry growth and yield will be recorded periodically to determine population resurgence by the nematodes and resulting effects on blueberry.

A NOVEL ASCAROSIDE CONTROLS THE PARASITIC LIFE CYCLE OF THE ENTOMOPATHOGENIC NEMATODE *HETERORHABDITIS BACTERIOPHORA*. **Noguez, Jaime<sup>1</sup>, E.S. Conner<sup>2</sup>, Y. Zhou<sup>1</sup>, T.A. Ciche<sup>3</sup>, J.R. Ragains<sup>2</sup>, and R.A. Butcher<sup>1</sup>. <sup>1</sup>Department of Chemistry, University of Florida, Gainesville, FL, 32611; <sup>2</sup>Department of Chemistry, Louisiana State University, Baton Rouge, LA, 70803; and <sup>3</sup>Department of Microbiology and Molecular Genetics, Michigan State University, East Lansing, MI, 48824.** 

Entomopathogenic nematodes survive in the soil as stress-resistant infective juveniles (IJs) that seek out and infect insect hosts. Upon sensing internal host cues, the IJs regurgitate bacterial pathogens from their gut that ultimately kill the host. Inside the host, the nematode develops into a reproductive adult and multiplies until unknown cues trigger the accumulation of IJs. Here, we show that the entomopathogenic nematode *Heterorhabditis bacteriophora* uses a pheromone to control IJ development. The pheromone, which likely increases in concentration at higher nematode densities, prevents IJ recovery to the J4 stage, allowing IJs to amass late in the infection process. Using activity-guided fractionation and NMR-based structure elucidation, we identify the chemical structure of the pheromone. The pheromone is structurally related to the dauer pheromone ascarosides that the free-living nematode *Caenorhabditis elegans* uses to control its development. However, none of the *C. elegans* ascarosides are effective in *H. bacteriophora*, suggesting that there is a high degree of species specificity. Our report is the first to show that ascarosides are important regulators of development in a parasitic nematode species.

### INCIDENCE OF PEACH TREE SHORT LIFE INCREASED BY FOLIAR NICKEL APPLICATION. Nyczepir, Andrew P. and B.W. Wood. USDA-ARS, SE Fruit & Tree Nut Research Laboratory, 21 Dunbar Road, Byron, GA 31008.

Peach tree short life (PTSL) is reportedly caused by a predisposition of trees to bacterial canker (*Pseudomonas syringae* pv. *syringae* van Hall), cold injury, or a combination of both, that is the consequence of root feeding by the ring nematode, *Mesocriconema xenoplax*. Certain micronutrients such as nickel (Ni) are effective in managing plant diseases caused by fungi, bacteria, or nematodes (*Meloidogyne* sp) and are also essential mineral elements. The ability of postplant nickel (Ni) foliar application to suppress *M. xenoplax* population density and thereby prolong survival of peach trees on a PTSL site was investigated from 2004-2011. Plots consisted of three treatments: i) Ni [foliar applied]; ii) methyl bromide fumigation (MBr); and iii) an untreated control. Peach trees ('Dixiland' on Nemaguard rootstock) were planted into all plots in March 2005 and the foliar Ni treatment was applied three times in 2005 and 2006. Nickel did not detectably suppress *M. xenoplax* populations as compared to MBr fumigation. The effectiveness of MBr fumigation, as measured by *M. xenoplax* population density, collapsed 27 months after orchard establishment. Trees receiving multiple foliar Ni applications at 0.45 g·L<sup>-1</sup> over two years, while exposed to *M. xenoplax*, exhibited greater ( $P \leq 0.01$ ) PTSL mortality than trees growing in untreated or MBr fumigated plots. These results suggest that foliar applications of Ni to peach trees, growing on a PTSL site, can disrupt tree metabolic/physiological processes sufficient to increase incidence of PTSL tree mortality and should therefore be used with caution in commercial orchards.

# NEMATICIDES EFFECTS ON *MELOIDOGYNE INCOGNITA* AND *ROTYLENCHULUS RENIFORMIS* IN COTTON FIELDS WITH VARIABLE SOIL TEXTURE. **Overstreet, Charles<sup>1</sup>**, **E.C. McGawley<sup>1</sup>**, **D. Xavier<sup>1</sup>**, **M. Kularathna<sup>1</sup>**, **M. Martin<sup>1</sup>**, **D. Burns<sup>2</sup>**, **R.L. Frazier<sup>3</sup>**, **and B. Haygood<sup>4</sup>**. LSU AgCenter, <sup>1</sup>Dept. of Plant Pathology and Crop Physiology, Baton Rouge, LA 70803; <sup>2</sup>County Agent, St. Joseph, LA 71366; <sup>3</sup>County Agent, Tallulah, LA 71282; and <sup>4</sup>DowAgroSciences, Collierville, TN 38017.

Nematicide types have changed dramatically during the past several years with the advent of seed treatment nematicides as the primary chemicals used by producers for dealing with cotton nematodes in the mid-South, U.S.A. Nematicide responses have recently been shown to be impacted by changing soil texture within the same field. A field study was conducted in 2011 to evaluate the response of a seed treatment nematicide (abamectrin) alone or in combination with 1,3-dichloropropene (1,3-D) at 28.1 l/ha and a no-nematicide control. The field had variable soil texture as measured by apparent electrical conductivity (EC<sub>a</sub>) and each treatment was replicated 20 times. Soil samples for analysis of nematode populations were collected prior to planting and after harvest. Population densities of root-knot nematode (Meloidogyne incognita) were significantly higher at harvest in both the no-nematicide and abamectrin treatments than in the treatment receiving 1,3-D. However, there were no significant differences in population levels among any of the treatments with reniform nematode (Rotylenchulus reniformis) after harvest. Yields were significantly greater for the combinations of nematicides or abamectrin alone treatments compared to the no-nematicide control, 1344, 1248, and 1127 kg/ha of cotton lint, respectively. Yields in all treatments increased with ECa-dp values. Trend for yield from the no-nematicide control, abamectrin, and combination of abamectrin and 1,3-D treatments ranged from 1025-1400, 1200-1380, and 1320-1410 kg/ha, respectively as EC<sub>a-dp</sub> readings increased from 18-90 mS/m. The no-nematicide treatment would require an  $EC_{a-dp}$  reading of 50 mS/m to yield as well as the abamectrin treatment at its lowest EC<sub>a-dp</sub> reading and a reading of 75 mS/m to be comparable to the combination of nematicides at the lowest ECa-dp value. The abamectrin alone would require an ECa-dp reading of 60 mS/m to be comparable to the combination of nematicides at the lowest EC<sub>a-dp</sub> value. Since seed treatment nematicides are likely to be planted across

#### 484 Journal of Nematology, Volume 44, No. 4, December 2012

an entire field, supplemental fumigants such as 1,3-D may be required in areas of the field with the lowest  $EC_a$  values, particularly in areas with concomitant populations of reniform and root-knot nematodes.

ENVIRONMENTAL FACTORS AFFECTING COMMUNITY STRUCTURE OF NEMATOPHAGOUS FUNGI AND THEIR PREY IN FLORIDA CITRUS GROVES. **Pathak, Ekta<sup>1</sup>, R. Campos–Herrera<sup>1,2</sup>, F.E. El–Borai<sup>1, 3</sup>, R.J. Stuart<sup>1</sup>, J.H. Graham<sup>1</sup>, and L.W. Duncan<sup>1</sup>. <sup>1</sup>Citrus Research and Education Center, University of Florida, 700 Experiment Station Rd, Lake Alfred FL 33850; <sup>2</sup>Instituto de Ciencias Agrarias, CSIC, Serrano 115 dpdo Madrid, 28006,Spain; and <sup>3</sup>Plant Protection Department, Faculty of Agriculture, Zagazig University, Egypt.** 

Entomopathogenic nematodes (EPNs) are important biological control agents whose efficacy depends upon various environmental parameters. Knowledge of how biotic and abiotic factors influence indigenous entomopathogenic nematode communities is necessary for rational management decisions. Nematophagous fungi (NF) are important natural enemies of nematodes but the role played by NF in the spatial distribution of indigenous EPNs is poorly understood. In this study, NF, EPNs and soil physical and chemical properties were examined in two Florida eco-regions. A survey of 53 citrus orchards in Florida's central ridge and flatwoods regions was conducted during two consecutive years. Seven species of NF (4 trapping NF, 2 endoparasites and an egg parasite) associated with nematodes were quantified directly using a real time qPCR assay. Soils were analyzed for different physical and chemical properties (a total of 41 properties; texture, water content, pH, nutrients, organic matter, pesticides, heavy metals and metaloids, etc). Only six of the abiotic properties were significantly different between the central ridge and flatwoods. Flatwoods soils had more magnesium, calcium and greater cation exchange capacity. The central ridge had higher elevation and greater levels of lead and manganese. All nematophagous fungi studied were frequently detected (24-56%) in the two regions except for Arthrobotrys musiform is present at  $\sim 10\%$  of sites and Hirsutella rhossiliensis detected at only two sites. NF diversity and evenness were higher in the flatwoods, whereas richness and relative abundance were similar for the two regions. *Paecilomyces lilacinus* and *Gamsylella gephyropagum* were encountered more frequently in the flatwoods (P = 0.03) and on the ridge (P = 0.02), respectively. Principle component analysis of all the environmental variables revealed 12 variables that explained 77% of the total variability. The first two axes of redundancy analysis (RDA) for NF explained 34.6% of the total variability. First and all canonical axes were significant (P = 0.01 and P = 0.006, respectively) in the resultant ordination. Potassium, copper, electrical conductivity, available water content and pH were positively related to NF whereas, sand and elevation were negatively correlated (P < 0.05). Thus, all but one NF species were associated with finer soils at low elevation (flatwoods habitats) in contrast to some EPNs (S. diaprepesi and H. zealandica). Nevertheless, we found little evidence that NF affect regionality of EPNs. RDA with EPNs as nominal and dependent variables and abiotic and NF (biotic) factors as independent variables revealed 44.5% variance in EPN distribution; however, none of the NF species explained the EPN spatial distribution.

MORPHOLOGICAL VARIATIONS IN THE MALE OCCURRENCE OF RENIFORM NEMATODE IN HAINAN ISLAND, CHINA. **Pham, T. Hoa<sup>1,2</sup> and J.W. Zheng<sup>1</sup>**. <sup>1</sup>Institute of Biotechnology, College of Agriculture & Biotechnology, Zhejiang University, Hangzhou 310058, P. R. China; and <sup>2</sup>LamDong Plant Protection Sub-department, 12- Hungvuong, DaLat, Vietnam.

The reniform nematode (Rotylenchulus reniformis) is considered one of the most economically important pests because it causes significant yield losses in cotton and other plants. This nematode is also recorded in the quarantine list with broad geographical distribution and host range. Surveys of the reniform nematodes have been continuously implemented to detect and prevent the spread of this nematode. In this study, soils around the rhizosphere roots of fruit trees and ornamental plants were collected in Hainan Island, China. Nematodes were extracted from 100 g soil by decanting and sieving techniques. The total numbers of individual nematodes, females and males of reniform nematodes were counted under light microscopes. According to Nakasono (2004), three types of reniform nematode populations based on observations of male occurrence were reported in Japan. The numerous-male type (MNT) revealed that the numbers of males and females were almost equal. The rare- male type (MRT) showed that rare or very small numbers of male occurred. The absent-male type (MAT) indicated only females were detected and males absent. Herein these three types of reniform nematode were also found in Hainan Island, China. Therefore, this study attempted to clarify any difference in the morphological variations of this nematode within these three biological types. The morphological data of R. reniformis populations in the present study were compared with previous studies and within three types. The morphometrics of immature females and males were similar to those from the same hosts in the published records, except a shorter tail length was found in this research. The MNT occurred at the highest frequency (62.5%), followed by the MRT (25%) and MAT (12.5%). Molecular techniques will be applied to discern the genetic variation of reniform nematodes from the different populations.

# THE EFFECTS OF PLANT PARASITIC NEMATODES ON ST. AUGUSTINE AND CENTIPEDE LAWNS IN LOUISIANA. **Plaisance, A.R. and E.C. McGawley.** Louisiana State University AgCenter, Department of Plant Pathology and Crop Physiology, Baton Rouge, LA 70803.

A survey was conducted to document management practices, agrichemical usage, foliar symptoms, pH, percent moisture, soil texture, and plant parasitic nematode communities in urban turf ecosystems. To date, 105 lawns in East Baton Rouge Parish have been sampled. A total of 11 nematode genera were identified from soil and root samples of St. Augustine lawns;

Criconemella have been found in 93%, Gracilicus in 1%, Helicotylenchus in 92%, Hemicycliophora in 1%, Hoplolaimus in 1%, Meloidogyne in 64%, Pratylenchus in 31%, Scutellonema in 1%, Tylenchorhynchus in 29%, Tylenchus in 98% and Xiphinema in 21%. Respectively, average nematode densities per 250cc of soil were 118, 20, 208, 11, 12, 51, 34, 11, 34, 128, and 11. A total of 10 nematode genera were identified from soil and root samples of centipede lawns; Criconemella have been found in 100%, Helicotylenchus in 88%, Hoplolaimus in 35%, Meloidogyne in 35%, Pratylenchus in 82%, Scutellonema in 1%, Trichodorus in 12%, Tylenchorhynchus in 18%, Tylenchus in 100% and Xiphinema in 18%. Respectively, average nematode densities per 250cc of soil were 286, 277, 22, 17, 63, 25, 9, 37, 129, and 14. The pH of the soils from St. Augustine lawns ranged from 5.1 to 6.9, with an average of 6.2. Centipede soil pH ranged from 6.1 to 7.3, with an average of 6.5. Percent soil moisture ranged from 7.7% to 25% with an average of 17.4% for St. Augustine and from 5.8% to 20.6% with an average of 15.4% for centipede. Soil temperatures during the summer ranged from 26.4°C to 34.3°C, with a sharp decline to 20°C in the fall. Using the soil hydrometer method of soil classification, types range from clay (50%+ clay content) to sandy clay loam (60% sand, 13% clay, 26% silt), to silt loam (10% sand, 11% clay, 79% silt), with the average being loam (30% sand, 20% clay, 50% silt). The most prevalent nematodes, ring, spiral, stunt, and lesion, have been isolated and established in axenic cultures for use in greenhouse pathogenicity trials. Microplot trials, currently in progress, will evaluate the impact of soil type on nematode reproduction and pathogenicity on these two grass species. Treatments in microplot trials include three soil types (clay loam [35% sand, 34% clay, 31% silt], sandy loam [70% sand, 13% clay, 17% silt], and sandy clay loam [55% sand, 22% clay, 23% silt]), three nematode community infestation levels (0, 1000 and 10,000 nematodes) and two grass species (St. Augustine and centipede). Soil will be infested with nematodes two weeks after transplanting 10 cm square sod pieces into microplots. Grass will be cut to a height of 2 cm every two weeks, and clippings will be dried and weighed. Soil subsamples will be collected monthly to evaluate nematode reproduction.

### "HEIRLOOM" TOMATOES AND ROOT-KNOT NEMATODES: HOST STATUS AND PLANT DAMAGE. Ploeg, Antoon, and Oli Bachi. Department of Nematology, University of California, Riverside, CA 92521.

Heirloom tomato varieties are popular with home gardeners because they are reported to have an "old-fashioned" tomato flavor and because of their appearance. A drawback to growing these varieties is that they are usually susceptible to diseases and pests, including root-knot nematodes. One way to circumvent the susceptibility to root-knot nematodes is to graft the scions on to *Mi*-gene nematode resistant rootstock. A field trial on a root-knot nematode (*Meloidogyne incognita*) infested site was done to evaluate the effects of grafting five "heirloom" tomato varieties on the resistant variety Hypeel45. The effects of grafting were different between the tomato varieties. In three of the five varieties, tomato yields were dramatically improved on grafted plants, whereas in the other two varieties in nematode host status and plant tolerance were evaluated in a subsequent greenhouse pot trial. One-gallon pots were inoculated with a 0, 1,000, 10,000 or 100,000 *M. incognita* eggs, and planted with 4-wk-old tomato seedlings. Nematode root populations and plant growth parameters were determined. Results from this trial confirmed that there are significant differences between heirloom tomato varieties in host status and tolerance for *M. incognita*. Data from these trials will be used as a basis for recommendations on nematode management strategies when growing heirloom tomatoes.

SPECIES BOUNDARIES IN CRICONEMATIDAE. **Powers<sup>1</sup>**, **Thomas O. and Ernest C. Bernard<sup>2</sup>**. <sup>1</sup>Department of Plant Pathology, University of Nebraska, 406 Plant Sciences Hall, Lincoln, NE 68583-0722; and <sup>2</sup>Department of Entomology and Plant Pathology, 213 Biotechnology Bldg., University of Tennessee, Knoxville, TN 37996-4560.

Taxonomic boundaries within Criconematidae have been considered as "taxonomically opaque". In spite of their distinct and sometimes dramatic morphology, the taxonomy of Criconematidae has been plagued by name changes, an abundance of synonymies, and surprisingly broad species definitions. According to the literature, numerous species have cosmopolitan distributions and exist in a wide range of ecosystems. We hypothesize that many of the presumed cosmopolitan species when compared across Major Habitat Types in North America will be revealed to be species complexes. We are testing this hypothesis using a combination of morphological and molecular analyses and a lineage species concept.

AN OVERVIEW OF THE ROTHAMSTED AND WAGENINGEN NEMATODE COLLECTIONS, TWO EUROPEAN COLLECTIONS OF INTERNATIONAL IMPORTANCE. **Prior, Thomas<sup>1</sup>, E. Van Heese<sup>2</sup> and G. Karssen<sup>2</sup>.** <sup>1</sup>The Food and Environment Research Agency, Sand Hutton, York, YO41 1LZ; and <sup>2</sup>National Plant Protection Organisation, Nematology, Geertjesweg 15, 6706 EA Wageningen, The Netherlands.

Following long-term changes at Rothamsted Research, the Rothamsted Nematode collection (RNC) has recently been transferred to The Food and Environment Research Agency (Fera). This historic collection comprises of approximately 16,000 slides of plant-parasitic nematodes, over 3,500 of which are type. The collection has now been extensively catalogued and is currently undergoing restoration, which includes digital image capture for a virtual web-based collection. Fera have retained the function of the RNC as the premier UK depository for slide material of all newly described nematode species, and continues to facilitate taxonomic research by offering specimens for loan. The Nematode Collection of Wageningen University (NCWU) has recently transferred to the National Plant Protection Organisation (NPPO), The Netherlands. This

collection, combined with the already existing slide collection of the NPPO, has been re-named the Wageningen Nematode Collection (WaNeCo). A searchable type specimens collection and nomenclatorial database has been created (www.waneco.eu). WaNeCo maintains nearly 3.600 accessions of types originating from terrestrial, freshwater and marine habitats worldwide, excluding parasites of higher Animalia. The nomenclatorial database is based on a former card system of about 25,000 cards of nominal taxa and synonyms, which have now been digitized. The regular slide collection includes about 50,000 slides, representing about 200,000 individual identified nematodes. The major part of this collection is focused on terrestrial nematodes from Europe. It is used for teaching, identification and taxonomic studies. Q-bank is the nematological approach of offering ecological, morphological and molecular information for the identification of regulated and other relevant plantparasitic nematodes. Both collections support this initiative to conserve existing knowledge but to also provide modern tools for nematode identification. This database comprises ecological, morphological, physiological, and sequence data of properly documented populations of more than 50 plant parasitic nematodes species, allowing plant protection organizations, inspection bodies and private laboratories an accurate identification. The main focus of Q-bank Nematodes is on European quarantine nematodes and economically important plant-parasitic nematodes from all over the world, however, for accurate distinction of regulated from non-regulated pests and diseases, descriptions of the latter are equally important. Special attention is paid to the genus Meloidogyne (root-knot nematodes), with more than 20 species (over 400 sequences) included. Q-Bank is updated by a team of curators with connections to relevant phytosanitary collections. Q-bank is supported by the Dutch Ministry of Economic Affairs, Agriculture and Innovation and by QBOL, an EU-project with partners from 20 countries (www.q-bank.eu/Nematodes).

HG TYPES AND RACES OF *HETERODERA GLYCINES* IN NORTH CAROLINA. **Quintero<sup>1</sup>**, **Tonia**, **Steve Koenning<sup>1</sup>**, **and Weimin Ye<sup>2</sup>**. <sup>1</sup>Department of Plant Pathology, North Carolina State University, Raleigh, NC 27695; and <sup>2</sup>Nematode Assay Section, Agronomic Division, North Carolina Department of Agriculture & Consumer Services, Raleigh, NC 27607.

A survey of North Carolina, South Carolina, and Virginia soybean production fields was conducted from 2010-2012 to determine prevalent species and races of plant-parasitic nematodes. An area-frame probability sampling design was used for field selection in order to avoid bias. Approximately 30 percent of the one hundred fields sampled in North Carolina were infested with detectable levels of *Heterodera glycines*. Only twenty of the populations could be adequately classified as to race or HG type. Some populations could not be increased to adequate levels for classification. Of classified populations twelve were race 2 or HG type 1.2.5.7. The peanut root-knot nematode was identified in three fields using PCR and perineal patterns. These data show that breeding for resistance to race 2 or HG type 1.2.5.7 should be prioritized in breeding programs.

AN ASSESSMENT OF SOIL HEALTH AND PRODUCTIVITY IN COMMUNITY AND MARKET GARDENS. **Reeves**, **Jennifer<sup>1</sup>**, **Z. Cheng<sup>2</sup>**, **M.T. Kleinhenz<sup>3</sup> and P.S. Grewal<sup>2</sup>**. <sup>1</sup>Environmental Science Graduate Program; <sup>2</sup>Entomology Dept.; and <sup>3</sup>Horticulture and Crop Science Dept., The Ohio State University, OARDC, Wooster, OH 44691.

The 2007 home foreclosure crisis has created a renewed interest in urban agriculture as the newly accumulated vacant land is being considered for increasing access to fresh produce in disadvantaged neighborhoods. However, concerns about the quality of urban soil and its suitability for food production arise from the physical and chemical disturbances associated with anthropogenic activity in the urban environment. This study investigated urban soil health through nematode community analysis and crop productivity through tomato fruit yield in community and market gardens within the city of Cleveland, Ohio, USA. We hypothesized that the market gardens would have higher soil health and crop productivity than the community gardens since market gardens are driven by profit motives to produce quality crops. Results indicate that, while the structure and enrichment of the nematode communities were similar between garden types, the market gardens had greater numbers of total nematodes ( $75.4 \pm$ 4.9 Vs 47.6  $\pm$  5.0, p=0.000), including bacterivores (47.6  $\pm$  3.9 Vs 28.3  $\pm$  3.4, p=0.001), omnivores (4.0  $\pm$  0.6 Vs 1.7  $\pm$  0.3, p=0.005), and plant parasitic nematodes ( $16.8 \pm 1.6$  Vs  $11.3 \pm 1.7$ , p=0.026) than the community gardens, indicating higher ecosystem productivity. Similarly, the NH<sub>4</sub> ( $3.3 \pm 0.2$  Vs  $2.2 \pm 0.3$ , p=0.002), NO<sub>3</sub> ( $37.8 \pm 3.3$  Vs  $20.6 \pm 2.8$ , p=0.000), Dissolved Organic Nitrogen ( $24.8 \pm 2.2$  Vs  $15.6 \pm 1.7$ , p=0.003) and Microbial Biomass Nitrogen ( $100.7 \pm 2.2$  Vs  $69.3 \pm 6.4$ , p=0.004) measurements were greater in the market gardens than in the community gardens. However, there was no difference of harvest (neither kg/m<sup>2</sup> nor kg/plant) between the market and community gardens. The nematode and soil N results support our hypothesis that market gardens have higher soil health than community gardens while productivity needs to be further investigated to understand why productivity is not reflecting these higher levels.

USING GENETICS AND GENOMICS TO CONTROL A NEMATODE-INFLUENCED DISEASE SYNDROME. **Reighard, Gregory<sup>1</sup>, X. Liu<sup>1</sup>, G.A. Swire-Clark<sup>1</sup>, W.C. Bridges<sup>2</sup>, A.G. Abbott<sup>3</sup>, C. E. Wells and W.V. Baird<sup>4</sup>. <sup>1</sup>School of Agricultural, Forest and Environmental Sciences; <sup>2</sup>Department of Applied Economics & Statistics; <sup>3</sup>Department of Genetics and Biochemistry, Clemson University, Clemson, SC 29634; and <sup>4</sup>Department of Horticulture, Michigan State University, East Lansing, MI 48824.** 

The "disease syndrome" Peach Tree Short Life (PTSL) involves the interaction of environmental conditions (winter temperature, soil history, early pruning) and pathogens ring nematode (*Mesocriconema xenoplax*) and *Pseudomonas syringae*.

PTSL is characterized by premature tree death by bacterial canker or cold injury, resulting in substantial economic losses, which have been observed in the Southeastern U.S. for the past 40+ years. Early research showed that rootstock affected PTSL incidence, and a collaboration of Clemson University and USDA-ARS lead to the release of a more tolerant rootstock trademarked Guardian® 'BY520-9'. To investigate the genetic and molecular basis for this tolerance, Nemaguard, a PTSL susceptible rootstock, and Guardian® selection 3-17-7 were crossed. The  $F_1$  plants were self pollinated to create segregating  $F_2$  populations. The trees were rated annually, from 2004 through 2008, for their response to PTSL. One hundred and seventy-six microsatellite/Simple Sequence Repeat (SSR) markers, each mapping to a specific chromosomal location on the *Prunus* reference nuclear genome, were used to screen the two parents and  $F_1$ -11. Only 53 markers showed polymorphism among the parents, and were heterozygous in  $F_1$ -11. These SSRs were then screened on the  $F_2$ -11 population (N=100). Segregation data for PTSL-response and SSR marker inheritance were complied and subjected to Analysis of Variance to determine differences in Genotypic Means (GMs). Nine SSR loci correlated with a response to PTSL. These nine SSR loci were distributed on four linkage groups (e.g., Linkage Group-1, LG-2, LG-4 and LG-6). Four of the nine loci (EPDCU5100, Pacita 27, UDA008 and UDA029) accounted for much of the variance in the trait based upon their large GMs (>2.5). After creating a molecular map from the segregation data, QTL analysis identified a quantitative locus on LG2 that was important in all five years of the study. In addition, this chromosomal interval contained three of the four SSR marker loci that accounted for the majority of the variation. These results identified regions of the peach genome where genes controlling PTSL susceptibility and tolerance reside, thus helping to define the genetic basis for response to PTSL as a complex but tractable trait. Disease resistant genes found in the region of the most promising PTSL-associated QTL will be discussed.

HOST RANGE ADDITIONS FOR *HETERODERA URTICAE* AND *CACTODERA BETULAE* FROM ARKANSAS. **Robbins<sup>1</sup>**, **Robert T., and Weimin Ye<sup>2</sup>**. <sup>1</sup>Plant Pathology Dept., University of Arkansas, Fayetteville, AR 72701; and <sup>2</sup>North Carolina Department of Agriculture & Consumer Services, Nematode Assay Section, 4300 Reedy Creek Road, Raleigh, NC 27607-6465.

In January, 2012, samples were taken from a variety of winter weeds at Toad Suck Park on the Perry County bank of the Arkansas River near Conway, Arkansas. Cysts of *Heterodera urticae*, that was first reported at the 2011 SON meetings, were present in large numbers. Critical inspection of these plant roots revealed white females attached to the roots of common chickweed (*Stellaria media* (L.) Vill. Numerous juveniles were found in the soil associated with chickweed, but males were not present. Several species of winter weeds were inoculated with juveniles obtained from cysts recovered from the soil to determine other possible hosts. Results of this test are still to be determined. A practical problem that could occur with *H. urticae* on weeds in soybean fields might be mis-identification as *Heterodera glycines*. In separate investigations, In the fall of 2011, cysts associated with *Amorpha fruiticosa* L., commonly known as false indigo-bush, were identified as *Cactodera betulae* using both morphological and molecular techniques. This is a new host for this nematode. The nematode (*C. betulae*) had been found as early as 1989 in this area along the White River near Beulah, AR by D.G. Kim, but efforts to identify it at the time were unsuccessful. This nematode has also been was reported in association with black locust (*Robinia pseudoacacia*) in the Fayetteville, AR vicinity by R. D. Riggs. *Amorpha fruiticosa* is a new host for *C. betulae*. Species identification of both *H. urticae* and *C. betulae* were confirmed by molecular analyses of the near-full-length small subunit rDNA gene, D2/D3 expansion segments of the large subunit rDNA gene and internal transcribed spacer.

### HIGH-THROUGHPUT GENOTYPING IN GENETIC MAPPING AND BREEDING FOR ROOT-KNOT NEMATODE RESISTANCE. **Roberts, Philip A., L.-B. Huynh, S. Wannamaker, J.E. Ehlers and T.J. Close.** University of California, Riverside, CA 92521.

Traditional breeding approaches to develop root-knot nematode (Meloidogyne spp.) resistant crop plants are often inefficient due to time and cost requirements for phenotyping progenies for resistance response. Screens for resistance rely on assays of nematode reproduction or indices of root-gall symptoms and may be unreliable even under controlled conditions. Resistance selection improvements lie in application of molecular marker and large-scale genotyping approaches for indirect selection of resistance loci. In the major crops such as corn, cotton, and soybean, private sector breeding is applying these systems, but in public programs these innovations are often unavailable. We developed a comprehensive molecular breeding (MB) system for cowpea (Vigna unguiculata), an important grain legume and fodder crop. Three root-knot nematode species (*M. incognita*, *M. javanica*, *M. arenaria*) are highly damaging to cowpea directly and as components of a disease complex with Fusarium wilt (Fusarium oxysporum f. sp. tracheiphilum), however effective resistance genes for these pathogens are available for cultivar improvement. A genotyping platform was developed with genome-wide EST-derived SNP markers identified from 17 cDNA libraries, first as an Illumina 1536-SNP GoldenGate genotyping assay and subsequently as a more flexible KBioscience KASPar system. The genotyping assay was applied to 12 RIL populations to construct individual and consensus genetic maps incorporating 1107 markers. RIL populations segregating for resistance were phenotyped in field and growth-pouch screens and resistance loci located by QTL mapping. The genome-wide SNP marker coverage enables both foreground (trait-based) and background selection, combining targeted resistance loci together with genome regions carrying favorable growth, yield and other stress tolerance traits. The high-throughput capability allows large numbers of individuals to be genotyped simultaneously at hundreds of loci for marker-assisted backcrossing (MABC) and recurrent selection (MARS) approaches in MB programs. A configurable workflow for applying MB tools of this system being developed as an Integrated Breeding Platform by the CGIAR- Generation Challenge Program will be discussed.

### GRAPEVINE NEMATODE MANAGEMENT WITH *PAECILOMYCES LILACINUS* ON THE CENTRAL CALIFORNIA COAST. **Sances, Frank<sup>1</sup>, B.A. Aglave<sup>1</sup>, S. Ockey<sup>2</sup> and M.B. Dimock<sup>2</sup>.** <sup>1</sup>Pacific Ag Group, 1840 Biddle Ranch Road, San Luis Obispo, CA-93401; and <sup>2</sup>Certis USA L.L.C. 9145 Guilford Road, Suite 175, Columbia, MD 21046.

With the implementation of the Montreal Accord of 2007 on restricting ozone depleting gases, as well as responses to increasing regulatory restrictions, the use of methyl bromide and other fumigants in agriculture has been on a steady decline. Effective and safe alternative treatments are being investigated with renewed interest, registered, and used in commercial grape production throughout California growing regions. The present study was conducted to determine the effectiveness of single and multiple year treatments of the Bio Nematicide, MeloCon WG for control of plant parasitic nematodes of Grapevine in central California. Melocon is a granular formulation containing spores of the fungus Paecilomyces lilacinus strain 251, a naturally-occurring beneficial fungus from soil. Experimental treatments of MeloCon were applied to replicate plots at 4 lb/acre alone and in combination with the organo-silicon surfactant, Break-Thru at 0.05% v/v, compared to the commercial standard Nemacure at 4 qt/acre. Several crop parameters were evaluated to compare efficacy of these treatments against naturally occurring resident nematodes in a commercial central valley vineyard. The research was conducted from 2009 to 2011 where applications of MeloCon was applied through the drip irrigation system. The major grapevine nematodes present were root-knot nematode (RKN), ring nematode, stubby root nematode, pin nematode. MeloCon in combination with Break-Thru treatment exhibited the lowest nematode population counts, comparable to the commercial standard Nemacure. In 2009, 2010, and 2011, this treatment showed an average reduction of 42%, 57%, and 65.7%, respectively, in total nematode species,. This reduction was superior to all other treatments including the commercial standard. Calculated Reproduction factors from RKN annual averages showed the UTC, Nemacure, Melocon, and Melocon with Break-Thru, increased through 2011 at 3.26, 0.88, 0.69, and 0.31, respectively. Ring nematode reproduction factors from these same four treatments were 0.43, 0.13, 0.22, and 0.03 respectively. There were no differences in vine vigor measurement during 2009, but 2010 onward, MeloCon in combination with Break Thru at 4lb/acre had consistently better vigor than both untreated and chemical standard treatments.

EXPRESSION OF HOST-DERIVED RNAi TARGETED TO A ROOT-KNOT NEMATODE PARASITISM GENE IN *NICOTIANA TABACUM*. Schweri, Kathryn<sup>1</sup>, G. Huang<sup>2</sup>, M.G. Mitchum<sup>3</sup>, T.J. Baum<sup>4</sup>, R.S. Hussey<sup>2</sup>, R. Lewis<sup>1</sup>, and E.L. Davis<sup>1</sup>. <sup>1</sup>Department of Plant Pathology, North Carolina State University, Raleigh, NC 27695; <sup>2</sup>Department of Plant Pathology, University of Georgia, Athens, GA 30602; <sup>3</sup>Division of Plant Sciences, University of Missouri, Columbia, MO 65211; and <sup>4</sup>Department of Plant Pathology and Microbiology, Iowa State University, Ames, IA 50011.

The parasitism genes of phytoparasitic nematodes encode effector proteins that are produced in specialized esophageal gland cells and secreted from the nematode stylet into host plant root cells. The secreted nematode effectors are differentially expressed during the parasitic process from root invasion through the initiation and maintenance of complex feeding cells by sedentary phytoparasitic nematode species. The 16D10 parasitism gene of the root-knot nematode (RKN) Meloidogyne incognita is expressed in the subventral esophageal gland cells early in the parasitic process and produces an effector protein that can interact with a SCARECROW-like transcription factor in the plant cell. Previous studies in Arabidopsis thaliana plants have shown that the 16D10 transcript can be silenced using host-derived RNA interference (RNAi), and that Arabidopsis plants that expressed 16D10 double-stranded RNA (dsRNA) were highly resistant to all four major RKN species. The goal of this project is to extend the 16D10-RNAi technology to a crop species, and cultivated tobacco (Nicotiana tabacum) was chosen because of its agronomic importance and relative ease of transformation. Two haploid lines of N. tabacum, TN90 and Hicks, were transformed with the same 16D10-RNAi constructs that were developed for Arabidopsis, and midvein tissue culture of mature leaves was used to produce double-haploids. T1 tobacco progeny were produced through self-fertilization. Egg production M. arenaria and M. incognita were significantly reduced in roots of T1 16D10-RNAi tobacco plants as compared to RKN reproduction in wild-type plants. The potential correlation of 16D10 small RNA expression levels in transformed tobacco with nematode infection severity is now under investigation. New constructs using different promoters and different introns for the RNAi hairpin construct are currently being transformed into both tobacco and Arabidopsis to potentially improve 16D10 small RNA production and further reduce RKN infection of tobacco roots.

CULTIVAR SUSCEPTIBILITY TO THE FUSARIUM WILT AND COMPLEX AND RACE CHARACTERIZATION OF *FUSARIUM OXYSPORUM* F. SP. *VASINFECTUM*. Scott, T.Z.<sup>1</sup>, K.S. Lawrence<sup>1</sup>, S.R. Moore<sup>1</sup>, K. Glass<sup>2</sup>, E. v Santen<sup>2</sup>. <sup>1</sup>Department of Entomology & Plant Pathology, 209 Life Science; and <sup>2</sup>Agronomy and Soils Department, 201 Funchess Hall, Auburn University, AL 36849.

Fusarium wilt of cotton is an economical damaging disease caused by *Fusarium oxysporum* f. sp. vasinfectum (FOV) and highly impacted by the root knot nematode (*Meloidogyne incognita*, RKN). Yield potential, resistant and tolerance of cotton

cultivars was determined by challenging the cultivars along with RKN- resistant M-315 and RKN-susceptible Rowden line in a field naturally infested with RKN and FOV. In 2010, Fusarium wilt incidence ranging from 16.3% to 0.4%. PhytoGen 367 WRF and Stoneville 5458 B2RF displayed the fewest Fusarium wilt symptoms. Canonical analysis indicated PhytoGen 367 WRF is resistant to RKN supporting low populations while producing the greatest yields in the trial. The standard susceptible cotton, Rowden, averaged 2,149 root-knot eggs per gram of root while the resistant cotton, M-315 supported only 88 eggs per gram of root. PhytoGen 367 WRF root-knot populations were similar to those of M-315. Comparatively, PhytoGen 565 WRF appeared tolerant to Fusarium wilt; this cultivar supported high populations of RKN while producing high yields. The drought in 2011 was less favorable to disease development and Fusarium wilt incidence peaked at 3%. The standard susceptible cv. Rowden averaged 241 root-knot eggs per gram of root while resistant M-315 supported 66 root-knot eggs per gram of root. Drought limited the nematode populations in 2011. Molecularly recognized races 1, 4, and 8 were isolated from our fields. Numerous novel genotypes were also identified which morphologically are FOV but do not match any known molecular strains. Of the 110 isolates tested, 69% were molecularly identical to race 1, 22% to race 4, and 7% to race 8. These different races were collected from all cotton cultivars with no preference of cultivar to any lineage of FOV. Lineage II was the most common group isolated in 2010 and lineage IV containing race 4 was most frequently isolated in 2011. These results indicate that the population of FOV in our fields are genetically diverse and the predominate lineages varies with the environmental conditions of each season.

THE EFFECT OF TRANSGENIC ENDOGENOUS DEFENSE ELICITORS IN *ARABIDOPSIS* ON ROOT-KNOT NEMATODES (*MELOIDOGYNE SPP.*). Sekora, David.<sup>1</sup>, A. Huffaker<sup>2</sup>, W.T. Crow<sup>1</sup>, F. Kaplan<sup>2</sup>, H.T. Alborn<sup>2</sup>, and T. Mekete<sup>1</sup>. <sup>1</sup>Entomology and Nematology Department, University of Florida, P. O. Box 110620, Bldg. 970 Natural Area Drive, Gainesville, FL 32611; and <sup>2</sup>United States Department of Agriculture, Agricultural Research Service Center for Medical, Agricultural and Veterinary Entomology: Chemistry Research Unit, 1700 SW 23rd Drive, Gainesville, FL 32608.

It is well established that manipulation of signaling genes regulating plant defense can have great effects on protecting plant systems from various fungal and herbaceous insect pests. However, similar progress in developing resistance to plantparasitic nematodes is still lacking. The goal of this project was to test the effect of recently characterized defense signaling genes for their ability to regulate interactions between plant roots and root-feeding nematodes. *Arabidopsis thaliana* was used as a model plant to study phenotypic root-knot nematode (*M. incognita* and *M. javanica*) infection and reproduction. Four different transgenic cultivars were used, with defense signaling genes either constitutively expressed or knocked out. Test plants were grown either on sterile plates on plant tissue medium or in a sand/soil mixture in a growth chamber. Prior to inoculating plates with *Meloidogyne spp.*, nematodes were surface sterilized with an antibiotic/antifungal cocktail to eliminate the any kind of soil-borne contamination. The plates were kept under controlled conditions in a growth chamber for 30 days before assay. The effect on the root-knot nematodes is currently being evaluated.

A KNIFE IN THE DARK: *MELOIDOGYNE* SPP. OF FLORIDA'S GOLF COURSES. Sekora, Nicholas S., W.T. Crow, and T. Mekete. Entomology and Nematology Department, University of Florida, P.O. Box 110620, Bldg. 970 Natural Area Drive, Gainesville, FL 32611.

*Meloidogyne* spp. can be notoriously difficult to diagnose in turf situations due to the unknown species composition and their undetermined impact on various turf grasses. A preliminary survey of 20 golf courses known to have *Meloidogyne* spp. infecting turf grasses were sampled to determine the species present at each site. Golf courses were selected from three areas of Florida (north-central, southeast, and southwest) and for using one of three widely used *Cynodon dactylon* cultivars (Champion, Tifdwarf, and TifEagle). Using a combination of RFLP with SspI and DraI enzymes of the COII mitochondrial region and genomic sequencing, *M. graminis* was detected at 19 of the sampled sites and *M. marylandi* was detected at a single site. No mixed *Meloidogyne* populations were observed at any of the sites sampled. Bootstrap analysis of the COII region of mitochondrial DNA indicated three separate groupings within *M. graminis* isolates. While these groupings are not distinct enough to separate them into individual species, they may indicate the presence of physiological races within *M. graminis*. The isolate identified as *M. marylandi* by RFLP was confirmed with sequencing of the COII mitochondrial region. This is the first confirmed report of *M. marylandi* in Florida. Future studies will focus on differences in damage on commercial turf grasses by *M. graminis* and *M. marylandi*.

ENTOMOPATHOGENIC NEMATODES: EFFECTS OF THE SOIL AGROECOSYSTEM ON BIOLOGICAL CON-TROL POTENTIAL. **Shapiro-Ilan<sup>1</sup>**, **David I., T. C. Leskey<sup>2</sup>**, **S. E. Wright<sup>2</sup>**, **I. Brown<sup>3</sup>**, and **L. Fall<sup>3</sup>**. <sup>1</sup>USDA-ARS, SE Fruit and Tree Nut Research Laboratory, 21 Dunbar Rd., Byron, GA 31008, <sup>2</sup>USDA-ARS, Appalachian Fruit Research Station, Kearneysville. WV 25430, <sup>3</sup>Department of Biology, Georgia Southwestern State University, Americus, GA 31709.

The soil agroecosystem affects entomopathogenic nematode (EPN) fitness, e.g., survival, foraging, and infection behavior, which has a profound effect on the biocontrol potential of EPNs. In this presentation we provide an overview of some of the biotic and abiotic components that affect EPN fitness. Additionally, we focus specifically on two biotic factors (strain

differences and relationships with other biotic agents) and two abiotic factors (soil moisture and foraging behavior). Nematode species or strains vary in their ability to survive in the soil and infect target pests. For example, we recently conducted a broad screening of EPNs for potential to control the plum curculio, *Conotrachelus nenuphar* (a major pest of stone and pome fruit in North America) and discovered major differences in virulence at different temperatures and in different soil types. EPN relationships with other soil biotic agents can be beneficial, neutral or detrimental. Recently we investigated phoretic relationships between EPNs and earthworms, and detected advantages in EPN pest control efficacy when earthworms were present (due to enhanced nematode dispersal). Soil moisture is considered a critical factor in achieving biocontrol efficacy with EPNs. In 2011, we conducted mini-plot field trials in West Virginia and Massachusetts to determine the ability of *Steinernema riobrave* and *S. feltiae* to control *C. nenuphar* at varying soil moisture levels. *S. riobrave* caused high levels of *C. nenuphar* mortality (more so than *S. feltiae*) and interestingly, in one of the trials, *S. riobrave* performed equally well in soil with or without irrigation. Elucidating EPN foraging behavior and host-finding cues will lead to greater understanding of nematode infection dynamics and result in improved biological control. We recently discovered that EPNs respond directionally to electrical fields; thus electrical fields in the soil may assist EPNs in navigation or host-recognition. Additional characterization of diverse soil biotic and abiotic factors and their impact on EPN fitness is necessary for the expansion of EPN biocontrol utility.

# PATHOGENICITY OF *TRICHODORUS OBTUSUS* ON ZOYSIAGRASS IN SOUTH CAROLINA. Shaver, J Bradly, P. Agudelo, and S.B. Martin. School of Agricultural, Forest, and Environmental Sciences, Clemson University, Clemson, SC 29365.

A population of Trichodorus obtusus was found infecting a stand of Empire zoysiagrass (Zoysia japonica) in Hampton County, South Carolina in 2011. Symptoms in the field included thin, chlorotic turf with short, stubby and necrotic roots. Soil samples from these areas showed T. obtusus to be associated consistently with symptomatic turf (range per 100 cc soil). A greenhouse study was conducted to determine the pathogenicity of the nematode on Empire zoysiagrass. Cores of 10.16 cm in diameter by 15.24 cm in depth were taken from the symptomatic stand of zoysiagrass, where initial densities averaged 80 individuals per 100 cm<sup>3</sup> soil. The top 2.54 cm of turf and thatch from each core was removed and set aside. The remaining soil was bulked, mixed, and packed into 10.16 cm x 15.24 cm columns. The original 2.54 cm layer of sod was replanted and allowed to grow for 140 days. At the end of the experiment, the average density of T. obtusus was 230 individuals per 100 cm<sup>3</sup> of soil, which indicates that Empire zoysiagrass is a suitable host for *T. obtusus*. Roots from each column were washed to examine symptoms. Roots were short and stubby and similar in appearance to those originally observed in the field. Towards the end of the study, the plants became prone to wilting and required more frequent watering. This population of T. obtusus was compared to other trichodorid isolates, including a T. obtusus isolated from 419 bermudagrass (Cynodon dactylon x C. transvaalensis), populations of Paratrichodorus minor from bermudagrass and centipedegrass (Eremochloa ophiuroides) from South Carolina, and two populations of T. obtusus from Florida. Morphological characterization was performed by measuring 20 adults from each population. Following morphological confirmation, molecular characterization was performed by sequencing a ca 1.7 kb region within the 18S rDNA. To our knowledge this is the first report of T. obtusus on zoysiagrass and the first report of the nematode in South Carolina.

TRACING CARBON FLOW THROUGH THE SOIL NEMATODE FOOD WEB: DO LONG-TERM BURNING PRACTICES AFFECT CARBON TROPHIC DYNAMICS IN THE TALLGRASS PRAIRIE? Shaw, E. Ashley<sup>1</sup>, K. Denef<sup>2</sup>, M.F. Cotrufo<sup>2,3</sup>, and D.H. Wall<sup>1,2</sup>. <sup>1</sup>Department of Biology, <sup>2</sup>Natural Resource Ecology Laboratory, and <sup>3</sup>Department of Soil and Crop Sciences, Colorado State University, CO 80523.

Soil carbon (C) dynamics, such as those occurring during litter decomposition, are crucial to global C cycling. Soil nematodes contribute to litter decomposition, but trophic interactions and C flow through the soil food web are not well understood. Land management practices, such as burning, impact decomposition processes, soil biotic communities, and soil C dynamics. Compared to unburned grasslands, annual burn of grasslands stimulates decomposition rates, changes the soil food web trophic structure and, thus, may impact soil food web's C cycling. We examine here how tallgrass prairie's annual burn affects C flow dynamics through the soil nematode food web during root litter decomposition. *Andropogon gerardii* root tissue, uniformly enriched in <sup>13</sup>C content, was used. In a randomized, replicated greenhouse study, the <sup>13</sup>C-labeled dead roots, placed in litter bags, were buried in soil collected from 2 management treatments: a) annually burned (AB) or b) unburned (UB) areas at the Konza Prairie Long Term Ecological Research site. Decomposition was assessed over 6 months at 8 destructive harvests. Microbes were extracted by phospholipid fatty acid (PLFA) procedure and their nematode consumers (fungivores, bacterivores, omnivores, predators) were water-extracted (Baermann Funnel technique). These biota were analyzed for biomass, microbial community composition, faunal trophic position, and C isotope signature of different communities, to trace root-C through soil trophic levels. Root litter mass loss did not differ between the treatments. However, there were differences in microbial communities and nematode trophic group abundances. Microbial abundance was significantly lower in AB. The microbial community structure changed during decomposition, with significant increases in fungal:bacterial ratios in

UB and gram-positive:gram-negative bacterial ratios in both management treatments. Total nematode abundance increased after root litter addition in UB and decreased in AB, and shifted over time of decomposition in both soils. Root litter-derived C was rapidly incorporated into microbial-C and nematode-C, but relative incorporation into different trophic groups varied temporally. By 3 days, root litter-C was incorporated into fungi, bacteria, and bacterivorous nematodes in both treatments, and in fungivorous nematodes in AB. This suggests that the fungal pathway is more dominant in AB soils, while the bacterial pathway is in UB. By 6 months, root litter-C was concentrated in higher nematode trophic levels in both soils. These results suggest that during decomposition of root litter, C proceeds by differing pathways through the soil nematode food web in fire managed tallgrass prairie systems.

## MORPHOLOGICAL AND MOLECULAR IDENTIFICATION OF *PRATYLENCHUS AGILIS* ASSOCIATED WITH WHEAT ROOTS FROM SHANXI, CHINA. **Shi, Hongli and J.W. Zheng.** Institute of Biotechnology, College of Agriculture & Biotechnology, Zhejiang University, Hangzhou 310058, P. R. China.

Root-lesion nematodes of the genus *Pratylenchus* Filipiev, 1936 are migratory endoparasites. They are among the most economically damaging plant-parasitic nematodes partially due to their wide host range and geographical distribution. The objective of this study was to identify *Pratylenchus* specimens collected from the rhizosphere of wheat in Shanxi, China to species through morphological and molecular studies. A total of 22 female specimens were examined, but males were not found. LM observation of females revealed that the key morphological characters agree with the type specimen of *P. agilis*. Body is short and somewhat stout, almost straight when heat-relaxed. Labial region is low and flattened. Stylet is 13.7-15.7 µm long with somewhat rounded knobs. Pharyngeal glands are ventrally overlapping intestine. Tail is tapering slightly, and terminus is mostly broadly rounded and unstriated. There was a minor difference among mormophetrics, maybe due to regional variation. Amplification of rDNA-ITS using the forward primer TW81 (5'-GTA GGT GAA CCT GCT GCT G-3') and the reverse primer AB28 (5'-ATA TGC TTA AGT TCA GCG GGT-3') yielded a fragment of 882 bp. BLAST results demonstrated that the sequence similarity between *Pratylenchus* specimen from Shanxi isolate and *P. agilis* isolate PagKL2 (FJ712888) is 98.6%. Both morphological and molecular data confirmed that the nematode is *Pratylenchus agilis*. To our knowledge, this is the first report of *P. agilis* from the rhizosphere of wheat.

USE OF SPIROTETRAMAT IN THE POST-PLANT MANAGEMENT OF ROOT-KNOT NEMATODE IN EGGPLANT AND PEACH. Shirley, Andrew<sup>1</sup>, A.P. Nyczepir<sup>2</sup>, P.M. Brannen<sup>1</sup>, and J.P. Noe<sup>1</sup>. <sup>1</sup>Plant Pathology Dept., University of Georgia, Athens, GA 30602; and <sup>2</sup>USDA-ARS, SE Fruit & Tree Nut Research Lab, 21 Dunbar Rd, Byron, GA 31008.

Historically peach production and IPM management of nematodes has relied almost solely on pre- and post-plant applications of nematicides in the southeastern United States. Currently Telone II is the primary preplant fumigant used by peach growers, since methyl bromide and fenamiphos, the only post-plant nematicide, are no longer available. There has recently been an interest in the development of post-plant nematicides. Movento (spirotetramat; a synthetic tetramic acid, Bayer CropScience) has shown some promising nematicidal effects and is currently being evaluated on peach in the Southeast. Movento is currently registered as a broad-spectrum insecticide on peach and is classified as a Group 23 lipid biosynthesis inhibitor. Two studies using Movento were conducted from 2011-2012 with Meloidogyne incognita infected eggplant and peach using various rates of spirotetramat. The first study with eggplant cv. 'BlackBeauty' was performed in an attempt to establish efficacious rates for the peach studies. The study consisted of three treatments: i) Movento (0.63 kg ai/h), ii) adjuvant control, and iii) a nematode control. Each treatment was replicated six times in a randomized complete block design. All plants were inoculated with 20,000 M. incognita eggs and treatments were applied 10 days later. Soil samples were collected 30 and 60 days after treatment application (DAT). At 60 DAT, number of nematode eggs and dry shoot and root weights were determined. Treatment with Movento resulted in lower ( $P \le 0.05$ ) M. incognita reproduction and greater dry shoot and root weights as compared to untreated controls. A similar experiment was completed with M. incognita and 'Lovell' peach seedlings, treatments included: i) two rates of Movento (0.42 and 0.63 kg ai/h) ii) water control, iii) nematode control, and iv) adjuvant control. Each treatment was replicated eight times in a randomized complete block. All plants were inoculated with 20,000 *M. incognita* eggs and treatments were applied after 10 days. Nematode assays and plant growth data were collected as previously described for the eggplant experiment. Treatment of peach with Movento at the 0.42 kg rate reduced ( $P \le 0.05$ ) numbers of *M. incognita* J2 in the soil at 30 DAT, but no differences were detected at 60 days. Both studies will be repeated.

#### SCREENING OF SOYBEAN MATERIALS FOR RESISTANCE TO RACE 3 and 4 OF SOYBEAN CYST NEMATODE. Shusen Liu, Qiao Yang, and Heng Jian. Department of Plant Pathology, China Agricultural University, Beijing 100193.

Soybean Cyst Nematode (SCN), *Heterodera glycines* Ichinohe, is a serious yield-limiting factor on soybean production in China. It is widely distributed throughout the Northeast and Huang-Huai-Hai Plains in China. The area infested by SCN reaches to 2.67 million hectares and the annual yield loss caused by the nematode is approximately 1.5 million tons in China. Use of resistant cultivars is one of the most effective means for SCN management. In this study, resistances of 388 soybean

materials to race 3 and 349 to race 4 were evaluated in pot experiments in greenhouse in 2010 to 2011. The resistant level was classified based on the index of parasitism, according to Schmitt and Shannon: resistance=0-9%, moderate resistant=10-30%, moderate susceptible=31-60% and susceptible >60%. The results showed there were 36 resistant and 23 moderate resistant materials to race 3 and 13 resistant and 16 moderate resistant to race 4, respectively. Interestingly, four materials had common resistance to both race 3 and 4 among all tested. The study also indicated that some resistant materials can suppress the development of nematodes. At 15 days after inoculation, white females were observed on susceptible materials, comparing with only J3 or J4 were observed on resistant varieties. In addition, some materials are useful for breeding in future.

IMPACTS OF SURFACE MINING RESTORATION EFFORTS ON SOIL-DWELLING NEMATODE COMMUNITIES IN THE APPALACHIAN REGION. **Smith, Haley S. and E.C. Bernard.** Entomology and Plant Pathology Dept., University of Tennessee, 2431 Joe Johnson Drive, Room 205, Knoxville, TN 37996-4500.

Coal is the largest component of energy required to power electrical plants. In the United States, approximately 62% of coal is mined using surface mining, a technique that destroys native ecosystems. Post-mining reclamation is required to decrease long-term environmental impacts associated with surface mining. Traditional restoration of post-mining sites in the southeastern U.S. consists of methods that discourage above and below-ground succession of native flora and fauna communities. Recent advances in mining restoration techniques proposed by the Appalachian Regional Reforestation Initiative (ARRI) have improved reclamation of post-mining sites above-ground by increasing native tree abundance and growth rate. The below-ground component of these reclaimed areas remains undescribed. Diverse soil nematode faunas have been shown to increase nitrogen uptake in hardwood seedlings, which may play a vital role in these nitrogen-limited, early successional systems. We addressed how traditional restoration methods used following the passage of the Surface Mining Control and Reclamation Act (SMCRA) approved by the Office of Surface Mining, as well as ARRI's Forestry Reclamation Approach (FRA), affect below-ground nematode communities. Soils were collected from mining restoration sites using both post-SMCRA and FRA approaches from three age groups: 0-3 years since reclamation, 4-8 years since reclamation, and 9 + years since reclamation, as well as unmined forest soils. Nematodes were extracted from these soils and identified to morphospecies. Soil pH was also determined. In addition, the effect of these nematode communities on plant survival and nutrient uptake in mined soils was determined by seedling biomass of plants grown in mining soils with nematode communities of varying diversity. Carbon and nitrogen ratios of these seedlings were measured to estimate the importance of nematode communities as bioremediators. Nematode biodiversity increased with soil age, with forest soils having the highest biodiversity. Bacterial feeders were the dominant feeding group. Plectid diversity was higher in the oldest age groups of both SMCRA and FRA soils than in younger soils. Soil restoration age, not restoration technique or nematode community, was the primary driver of seedling biomass.

# COTTON IMPROVEMENT BY ALIEN INTROGRESSION OF RENIFROM NEMATODE RESISTANCE FROM *GOSSYPIUM LONGICALYX*: AN OVERVIEW. **Stelly<sup>1</sup>**, **David M., X. Zheng<sup>1</sup>**, **A. A. Bell<sup>2</sup>**, **A. Van Deynze<sup>3</sup>**, **H. Ashrafi<sup>3</sup>**, **and R. L. Nichols<sup>4</sup>**. <sup>1</sup>Texas A&M University, College Station, TX, <sup>2</sup>USDA-ARS-SPARC, College Station, TX, <sup>3</sup>University of California, Davis, CA, and <sup>4</sup>Cotton Incorporated, Cary, NC.

In the USA, the reniform nematode (Rotylenchulus reniformis) is estimated to cause over \$100M in annual losses to Upland cotton (Gossypium hirsutum) production. Genetic resistance could significantly benefit US growers. Moreover, the removal of chemicals from the market for nematode control is accentuating the need for genetic resistance to cotton nematodes. Years ago, screening of cotton germplasm collections revealed no high resistance to reniform nematodes among 52-chromosome Gossypium species, but revealed very high resistance in the 26-chromosome cotton relative G. longicalyx, a spindly African relative (2n=52). Good resistance levels were noted for other 26-chromosome species, too. Innovative breeding and collaborative efforts helped us circumvent ploidy and genomic barriers to introgression of the African species' resistance. We subsequently used breeding materials to localize and map the introgressed gene (*Ren<sup>lon</sup>*) to chromosome-11, and to establish a system for marker-assisted selection that contributed significantly to the development LONREN-1 and LONREN-2, two highly resistant lines that were jointly "released" to the public. Subsequent field-tests, however, revealed that the LONREN lines suffer from variable levels of "stunting", and that stunting was very severe in some locations. Various tests suggest that the severity of stunting is associated with nematode population density, and that LONREN lines are hypersensitive to the nematode combined with other soil pathogens. Several lines of evidence also indicate that genes linked to the introgressed gene (Renlon) affect resistance to other pathogens, and others affect field and fiber performance. Genetic dissection and characterization of this region is desirable for scientific and practical reasons. Towards these ends, we have mounted a map-based approach for high-resolution recombination, mapping, and minimization of the alien segments flanking the resistance gene. Our hypotheses regarding the "stunting" and approaches to analyze it will be discussed, including our breeding strategies, efforts to develop new sequence-based markers in the flanking region and the strategies to recover informative recombinants.

*GOSSYPIUM* ACCESSIONS RESISTANT TO *ROTYLENCHULUS RENIFORMIS* VARY IN SENSITIVITY TO THE HERBICIDE FLUOMETURON. **Stetina<sup>1</sup>**, **Salliana R. and W.T. Molin<sup>2</sup>**. <sup>1</sup>USDA ARS, Crop Genetics Research Unit, PO Box 345, Stoneville, MS 38776, <sup>2</sup>USDA ARS Crop Production Systems Research Unit, PO Box 350, Stoneville, MS 38776.

Reniform nematode (Rotylenchulus reniformis) resistance is being transferred to Upland cotton (Gossypium hirsutum) from its distant relatives. Anecdotal observations of fluometuron damage to LONREN lines with resistance from G. long*icalyx* raised concerns about introducing herbicide sensitivity from other resistance sources. The research objective was to evaluate fourteen sources of reniform nematode resistance for their reaction to fluometuron in a replicated greenhouse trial: G. herbaceum accessions A1-017 and A1-024; G. arboreum accessions A2-083, A2-100, A2-190, and A2-194; G. barbadense accessions Pima PHY 800, GB 713 and TX 110; G. hirsutum accessions T19, T1347, and T1348; and three G. hirsutum lines with resistance introgressed from G. barbadense (FR-05) or G. longicalyx (LONREN-1 and LONREN-2). The control was G. hirsutum cultivar Deltapine 161 B2RF. Six seeds of each line were planted on top of a mixture of sandy loam soil and sand (3:1 by volume) in 10 cm square pots. Fluometuron added to 100 cm<sup>3</sup> additional soil mix (1:1, sandy loam:sand) at rates of 0, 0.34, 0.67, 1.01, 1.34, and 1.68 kg a.i./ha was used to cover the seeds, and care was taken during watering to avoid disturbing soil. Three weeks after planting, each plant was scored for herbicide damage on scale of 0 to 4 where 0 = no damage and 4 = maximum damage observed, and electron transport rates (photosynthesis) in the cotyledons of two plants per pot were measured. Green tissue above the cotyledons was harvested from all plants in each pot and a combined dry weight determined. ANOVA compared accessions, regression determined the nature of the response to the herbicide, and contrasts compared response trends for accessions with the control. Damage ratings were higher on the G. arboreum accessions, while G. barbadense GB 713 and TX 110 showed less damage than the control. Damage increased linearly with increasing herbicide rate for all accessions. Gossypium arboreum A2-083 showed a greater increase in damage in response to increasing herbicide rates than the control, but no other accessions differed. All lines exhibited linear reductions in biomass as herbicide rates increased, but only the G. arboreum accessions and G. herbaceum A1-017 weighed less than the control. Gossypium barbadense GB 713 and Pima PHY 800 showed a greater magnitude in biomass reduction than the control, but no other accessions responded differently. Electron transport rates of all G. herbaceum and G. arboreum accessions and G. barbadense Pima PHY 800 were lower than the control. The relationship between herbicide rate and photosynthetic activity was curvilinear, with similar decreases in photosynthetic activity in response to increasing herbicide concentration for all accessions. Increased sensitivity to fluometuron could be introduced through wide crosses, but with the exception of G. arboreum A2-083, the accessions did not respond to the herbicide differently from the commercial control.

### *GLYCINE* ACCESSIONS EVALUATED FOR RESISTANCE TO *ROTYLENCHULUS RENIFORMIS*. Stetina, Salliana R., J.D. Ray, and J.R. Smith. USDA ARS Crop Genetics Research Unit, P.O. Box 345, Stoneville, MS 38776.

Identification of resistance to reniform nematode (Rotylenchulus reniformis) is the first step in developing resistant soybean (Glycine max) cultivars that will benefit growers in the Mid South, where soybean acreage on reniform nematodeinfested fields has increased in recent years. Seventy-five domestic and wild soybean (*Glycine max* and *G. soja*) lines were evaluated for resistance to infection by reniform nematode in a series of growth chamber tests. Each entry was evaluated in at least two tests using a completely randomized design with 5 replications. A single plant of each soybean line was established in a container filled with 120 cm<sup>3</sup> of a steam-sterilized soil mixture. Four weeks after 1,000 vermiform reniform nematodes were added to the soil, the number of swollen females attached to the roots was determined. Classification of entries was based on the percentage of infection as compared to the susceptible genotypes Morsoy RTS4706N (sets 1 and 3), Delta King DK4968 (sets 1, 2, and 3), Braxton (sets 4 and 5), and PI 88788 (sets 4 and 5): nematode index <10% = resistant, 10-30\% = moderately resistant, 31-60% = moderately susceptible, and >60% = susceptible. Both relative infection and consistency of phenotype across tests contributed to identification of the best materials. This project is the first to report on the reaction of 36 genotypes to reniform nematode. Of these, eight with moderate resistance were identified: released germplasm lines DS-880 and DS4-SCN05; accessions PI 417077, PI 507354, and PI 567516 C; and breeding lines DS97-84-1, 02011-126-1-1-2-1, and 02011-126-1-1-5-1. The remaining 28 previously untested lines were classified as moderately susceptible or susceptible to the reniform nematode. In a separate experiment, reniform nematode reproduction was evaluated on a subset of the lines originally tested to confirm their response: PI 90763, PI 230977, PI 417050, PI 567516 C, DS-880, DS97-84-1, DS4-SCN05, 2011-126-1-1-2-1, and 2011-126-1-1-5-1. This growth chamber experiment was conducted 3 times. Eight weeks after soil infestation with1,000 vermiform reniform nematodes, nematodes extracted from soil plus eggs extracted from roots were counted for each entry and compared to susceptible lines Braxton and PI 88788 and resistant lines Hartwig and PI 437654 using ANOVA and differences of least squares means. A fallow treatment was included to monitor survival of the nematode with no plant roots present. PI 90763, PI 567516 C, DS-880, DS97-84-1, DS4-SCN05, 2011-126-1-1-2-1, and 2011-1-1-5-1 consistently suppressed reniform nematode populations to levels comparable to those that developed on the resistant controls. Further, in two of the three tests, the reniform nematode populations on all of these lines were equivalent to or smaller than the population that persisted in the fallow pots. Infection-based screening results indicated that PI 230977 was moderately resistant and PI 417050 was moderately susceptible. However, both of these lines were grouped with the susceptible controls when infection, reproduction, and survival of the nematodes contributed to the classification.

RESPONSE OF CUCURBIT ROOTSTOCKS FOR GRAFTED MELON (*CUCUMIS MELO*) TO SOUTHERN ROOT-KNOT NEMATODE, *MELOIDOGYNE INCOGNITA*. **Thies, Judy A.<sup>1</sup>**, **Jennifer J. Ariss<sup>1</sup>**, **Sharon Buckner<sup>1</sup>**, **Richard L. Hassell<sup>2</sup>**, **and Amnon Levi<sup>1</sup>**. <sup>1</sup>U.S. Vegetable Laboratory, USDA, ARS, Charleston, SC; and <sup>2</sup>Clemson University, Charleston, SC.

Root-knot nematodes (RKN) are an important re-emerging pest of melon (Cucumis melo), due largely to the loss of methyl bromide as a pre-plant soil fumigant. Melon is highly susceptible to southern RKN, Meloidogyne incognita, which causes severe root galling and reduced melon fruit yields. Cucurbit rootstocks have been used to control soilborne diseases such as Fusarium wilt in grafted melon in Asia since the 1920's. During the last decade, grafting melon on resistant rootstocks has been considered a potential alternative to methyl bromide for managing soilborne diseases and pests on this crop in the U.S. We evaluated the response of commercial and wild-type cucurbit rootstocks representing different cucurbit species to *M. incognita* in greenhouse and field tests. *Greenhouse test*. In a greenhouse evaluation of 23 commercial cucurbit rootstocks and Plant Introductions (PIs) that can be used for grafting melon, PIs of African horned cucumber, Cucumis metulifer, exhibited the least (P<0.05) root galling among four cucurbit rootstock taxa with root gall indices (GI) ranging from 2.6 to 3.3, using a 1.0 to 5.0 scale where 1=0% to 3% root system galled and 5=80% to 100% root system galled. The Cucumis melo commercial melon rootstocks 'Dinero' and WR-15006 were highly susceptible with GI= 4.9 and 4.7, respectively. Likewise, three commercial Cucurbita moschata rootstocks and one PI (BS1, TZ 148, AQ, and PI 53009, respectively) were also susceptible with GI ranging from 4.5 to 4.7. Twelve Cucurbita maxima x C. moschata commercial squash hybrid rootstocks had GI ranging from 4.2 for 'Carnivor' to 5.0 for Jing Xin No.3. The standard Cucurbita maxima x C. moschata hybrid 'Strong Tosa' also was highly susceptible with GI=4.9. Field test, Charleston, SC. The scion 'Athena' melon (C. melo) was grafted on eight different cucurbit rootstocks and evaluated in a field that was highly infested with M. incognita. Rootstocks of Benincasa hispida, Cucurbita maxima x C. moschata, Cucurbita argyrosperma, C. melo, C. maxima, and Cucurbita *ficifolia* were all highly susceptible to RKN with numbers of *M. incognita* eggs per gram fresh root ranging from 659 for B. hispida to 6,793 for C. argyrosperma. Cucumis metulifer exhibited moderate resistance and supported 323 eggs per gram fresh root. Thus, C. metulifer was the only cucurbit rootstock evaluated in our tests that exhibited resistance to M. incognita. Currently, we are evaluating all of the C. metulifer PIs in the USDA Plant Introduction collection in order to identify the most resistant and vigorous accessions for use in developing RKN-resistant rootstocks for melon.

USE OF PROPAGATED PLANT CUTTINGS TO ACCELERATE SCREENING CAYENNE PEPPER FOR RESIS-TANCE TO *MELOIDOGYNE INCOGNITA*. **Thomas, Stephen<sup>1</sup>**, **J.M. Beacham<sup>1</sup>**, **and P.W. Bosland<sup>2</sup>**. <sup>1</sup>Department of Entomology, Plant Pathology and Weed Science, P.O. Box 30003 MSC 3BE; and <sup>2</sup> Department of Plant and Environmental Sciences, P.O. Box 30003, MSC 3Q, New Mexico State University, Las Cruces, NM 88003.

Using sodium hypochlorite to assess egg production per unit of root weight in young plants is the most sensitive, rapid, and quantitative approach for screening breeding lines for resistance to southern root-knot nematode (Meloidogyne incognita). However, a limitation to this approach is that it destroys those very plants identified as desirable for further investigation. In 2009, a greenhouse study was conducted to assess and enhance resistance to *M. incognita* in three NMSU cayenne chile (Capsicum annuum) cultivars, 'NuMex Nematador' (96 plants) and two accessions of 'NuMex Las Cruces' (79 & 78 plants each). Individual plants from each cultivar and from the standard resistant control 'Carolina Cayenne' were inoculated with 2,500 M. incognita eggs per plant and evaluated 42 days later. Prior to bleach extraction of roots, cuttings were propagated from each plant. No eggs (zero nematode reproduction) were recovered from two of 96 Nematador plants, and 1-5 eggs were recovered from four of 157 'NuMex Las Cruces' plants. Seeds from the two Nematador plants were collected separately and 24 progeny of each were tested in an additional greenhouse study in 2010, where some *M. incognita* reproduction was observed. In 2011, despite the results from previous years, both Nematador lines were transplanted into 90-cm diameter field microplots, along with cayenne cultivars 'Mesilla' (RKN-susceptible), 'Large Red Thick' (Nematador parent), and 'Carolina Cayenne'(resistant control). Half the plots had been pre-infested with M. incognita. Plants were fertilized and irrigated as needed throughout the season. Pod count, total pod weight, and RKN eggs/g dry root were recorded at harvest. One Nematador isolate showed the least RKN reproduction (4,533 eggs/g dry root) compared to susceptible Mesilla (78,689 eggs/ g dry root). Pod yield and weight were similar between infested and non-infested plots, with no evidence of pathogenicity attributable to nematode infection or host resistance response. The use of propagated plant cuttings maintains direct linkage between initial screening results for resistance to M. incognita and the deployment of such traits within time frames as short as two years.

SUPPRESSION OF *MELOIDOGYNE INCOGNITA* BY *PAECILOMYCES LILACINUS* IS ENHANCED BY PLANTING COVER CROPS. **Timper, Patricia<sup>1</sup> and G. Parajuli<sup>2</sup>**. <sup>1</sup>USDA ARS, P.O. Box 748, Tifton, GA 31793; and <sup>2</sup>Wageningen University and Research Center, 6708 PB Wageningen, The Netherlands.

*Paecilomyces lilacinus* is a common soil saprophyte and some strains of this fungus are aggressive parasites of sedentary stages of nematodes. The fungus is registered in the U.S. under the trade names MeloCon WG and NemOut. Persistence of *P. lilacinus* is relatively low in sandy soils compared to other soil types. Addition of organic matter to sandy soil was shown

to increase persistence of the fungus. In conventional agriculture, winter cover crops are used to reduce soil erosion and loss of plant nutrients. We hypothesized that, compared to fallow soil, growing a cover crop before application of *P. lilacinus* would increase efficacy of the fungus against *Meloidogyne incognita* on cotton. A greenhouse experiment was conducted in which cover crops were grown for 1 month, killed with herbicides, and the above-ground residue cut and left on the soil surface or removed. There were five cover crop treatments: 1) fallow, 2) rye + residue, 3) rye, no residue, 4) crimson clover + residue, and 5) crimson clover, no residue. The NemOut treatments (0 and 336 g/ha) were applied with a surfactant to a trench in the center of the pots. A single cotton seed was planted in the center of the trench cover with soil; the plants were inoculated with J2 of *M. incognita* 2 wks later. The experiment was conducted two times with seven replications per treatment. Nematode reproduction was assessed 60 days after inoculation. Percentage suppression of nematode suppression in the presence of residue was 60% for rye and 49% for clover compared to 35% in fallow soil. In the absence of residue, nematode suppression of *M. incognita* by *P. lilacinus* was improved when a cover crop treatments than in the fallow soil. In conclusion, suppression of *M. incognita* by *P. lilacinus* was left on the soil surface.

INHERITANCE OF RESISTANCE TO *MELOIDOGYNE JAVANICA* ROOT-GALLING IN A BRAZILIAN SOYBEAN. **Vinholes, Patricia da Silva<sup>1,2</sup>, V.M.P. Silva<sup>3</sup>, T. Dalla Nora<sup>3</sup>, I. Schuster<sup>3</sup>, A. Borem<sup>2</sup>, and P.A. Roberts<sup>1</sup>. <sup>1</sup>Departament of Nematology, University of California, Riverside, Riverside, CA, 92521; <sup>2</sup>Department of Plant Science, University of Vicosa, Vicosa, MG/Brazil; and <sup>3</sup>Cooperativa Central de Pesquisa Agricola, Cascavel, PR/Brazil.** 

In Brazil, root-knot nematode Meloidogyne javanica causes severe yield loss of soybean [Glycine max (L.) Merril] in many agricultural areas. An effective strategy to prevent yield loss is planting root-knot nematode resistant cultivars. However, Brazilian cultivars have narrow genetic base for resistance to root-knot nematode, derived from the cultivar Bragg. Inheritance of resistance to Meloidogyne javanica (Mj) induced root-galling in cv. MG/BR46 Conquista was analyzed in progeny developed from the cross MG/BR46 Conquista x CD204 (susceptible). One-hundred and forty F2:3 families and both parents were phenotyped for Mj galling reaction in a greenhouse experiment. Five plants per family were planted in conetainers and after 10 days each plant was inoculated with 5000 Mj eggs. Thirty days after inoculation root-galling severity per plant was scored using an index from 1-5, where 1 = less than 10% of roots with small galls; 2 = 10-25% of roots with small galls; 3 = 26-50% of root with galls; 4 = 51-90% of roots with large galls and 5 = 91-100% of roots with large galls and root rot. Families with mean gall score of 1-2 were considered resistant (R), 2.1-3.0 were moderately resistant (MR), 3.1-4.0 were moderately susceptible (MS), and 4.1-5.0 were susceptible (S). Among the F<sub>2:3</sub> families, 7 were R, 25 MR, 93 MS, and 15 S. Chi-square tests of different segregation ratios gave the best fit to a 12S+MS:3MR:1R ratio ( $\chi^2 = 0.49$ ; P = 0.78), supporting a model of resistance controlled by two recessive genes with epistatic effects. The predicted genotypes were 1R (aabb), 3MR (aaB), and 12 MS + S (A bb + A B). Complete resistance (R) to Mj root-galling was determined by two recessive genes (aabb), with one of them having larger effect resulting in the MR phenotype in plants containing only this gene. The other gene appeared completely epistatic, with plants containing only this gene being MS or S. Although this resistance was expressed quantitatively, its control by two genes with large combined effect provides a simple system for marker development for breeding.

GENETIC AND PHYSICAL ANALYSIS OF *MELOIDOGYNE INCOGNITA* RESISTANCE GENES ON AN INTER-SPECIFIC *GOSSYPIUM BARBADENSE* x *G. HIRSUTUM* PROGENY. **Wang, Congli<sup>1</sup>, M. Ulloa<sup>2</sup>, and P.A. Roberts<sup>1</sup>.** <sup>1</sup>University of California, Riverside, CA 92521; and <sup>2</sup>USDA-ARS, Cropping Systems Research Laboratory, Lubbock, TX 79415.

The root-knot nematode (RKN, *Meloidogyne incognita*) resistance gene *rkn1* in *Gossypium hirsutum* Acala NemX interacts with a transgressive factor *RKN2* from susceptible *G. barbadense* Pima S-7 to produce high resistance to RKN. The *rkn1* and *RKN2* genes are clustered and linked to SSR markers CIR316 and MUCS088, which are located on the telomeric region of chromosome (Chr) 11. QTL analysis on an  $F_{2:7}$  (Pima S-7 x Acala NemX) population validated the importance of this telomeric region, which contributed to resistance to both root-galling and nematode egg production. Of 48 SSR markers screened from Chr11, 29 SSRs amplified products located on homoeologous Chr21 with different size-alleles from those on Chr11. Marker allele-sizes were used to extract BAC clones from pools and super pools of Acala N901 (Acala NemX background) library. Preliminary blast analysis and sequence composition of 48 markers and 48 assembled BAC sequenced-clones of Acala N901 associated with the telomeric RKN resistance region indicated the existence of many copies of resistance gene analogs (RGA). One of two RGA sequences of CIR316\_222 (bp) (3148 bp) on Chr11 (32% identity to a potato late blight putative resistance RGA1 gene) had 83% identity with another RGA of CIR316\_214 (3375 bp) on Chr21. When CIR316\_222 and CIR316\_214sequences were compared with the corresponding region of the D5 *G. raimondii* genome sequence, the D5 sequence shared 88% identity with Chr11 and 92% identity with the RGA on Chr21. These sequence comparisons provided further insight into the organization and molecular evolution of the RKN-resistance gene cluster on Chr11 and its homoeolog Chr21.

MOLECULAR NEMATODE COMMUNITY ANALYSIS IN HAWAII. Wang, I.-C., K.-H. Wang, and Brent. S. Sipes. Dept of Plant and Environmental Protection Sciences, University of Hawaii at Manoa, Honolulu, HI 96822.

Nematodes are excellent indicators for soil health. However performing nematode community analysis is laborious and technically challenging. This research seeks to develop a qPCR-based molecular tool for nematode community analysis. qPCR detecting 18S rDNA offered one approach to identify and quantify free-living nematodes. Owing to too many unpredictable nematode genera across soil ecosystems, one strategy is to develop universal qPCR markers selective for key nematode guilds (Ba1, Ba2, F2, P4, Om4, Om5, and P5) that are most critical for nematode faunal analysis. Universal qPCR primers were successfully being identified for all of these guilds except for Ba1. Two primers were needed for F2; Om4, Om5, and P5 cannot be differentiated and were thus being combined as Om4/ Om5/ P5 by one primer. These primers were then verified by BLAST and then run through artificial nematode mixture sample composed with known nematode guilds. The results confirmed the validity of these universal primers. The next logical step was to run these qPCR to nematodes collected from four natural ecosystems: forest, organic, pineapple field, and beach sites. Visual nematode identification on these four systems was being conducted to compare results. Two qPCR standard curves (plasmid DNA and genomic DNA) were used to obtain nematode abundance of the four ecosystems. Since both DNA standard curves did not estimate nematode abundance comparable to the visual count, ranking of nematode community indices of the four ecosystems were compared between molecular and the visual methods. While the ranking calculated by the plasmid DNA standard curve of qPCR assay were not consistent with most of the nematode community indices calculated by visual method, 4 out of 8 nematode indices estimated by the gDNA standard curve were relatively consistent. This research provided universal nematode guild qPCR primer sets and initial protocol of qPCR-based molecular tool for soil nematode community analysis. Further research need to be conducted on better estimation of nematode abundance, richness and diversity. More universal primers selective for Ba1, Ba3, F3, P3, also Om4, Om5, and P5 individual primers are needed.

COVER CROPPING SYSTEM AND COMPOST TEA TREATMENT FOR MANAGEMENT OF NEMATODE, WHITEFLIES, AND POLLINATORS. **Wang, Koon-Hui<sup>1</sup>**, and **T. Radovich<sup>2</sup>**. <sup>1</sup>Department of Plant and Environmental Protection Sciences and <sup>2</sup>Department of Tropical Plant and Soil Sciences, University of Hawaii at Manoa, 3050 Maile Way, Honolulu, HI 96822.

A field trial was conducted in the spring (Trial I) and repeated in the late summer (Trial II) of 2011 at Twin Bridges Farm, Waialua, HI to evaluate the effect of strip-till planting of sunn hemp (Crotalaria juncea) or crimson clover (Trifolium incarnatum) cover crops in a zucchini cropping system. Alternate strips of cover crops served as living mulch intercropping with the cash crop. In addition, effect of chicken manure based compost tea was also tested. This was a split-plot experiment with sunn hemp, crimson clover, and bare ground as the main plot treatment, and organic fertilizer (=Fert; chicken pellet), compost tea (CT), organic fertilizer plus compost tea (Fert+CT), and none serve as subplot treatment. Due to poor reestablishment of crimson clover in Trial II, only SH and BG were tested in this trial. Similar results were observed between the two trials. Planting of SH significantly increased (P < 0.05) bacterivorous nematodes and suppressed plant-parasitic nematodes toward the end of the crop as compared to BG. However, planting of sunn hemp did not enhance the numbers of omnivorous nematodes in both of the zucchini cropping cycles. Crimson clover did not enhance beneficial nematodes nor suppressing plant-parasitic nematodes as compared to the BG. Numbers of the key plant-parasitic nematode at this site, reniform nematode (Rotylenchulus reniformis) were lower in Fert+CT than Fert only at the initial stage of the zucchini planting. Adding CT also increased % omnivorous nematodes more than Fert only treatment at the end of the crop. Planting of SH as living mulch increased the number of pollinators (carpenter bee and leaf cutter bee) encountered during a walk through as compared to BG. As living mulch, SH acted as a trap crop for silver leaf whiteflies, thus reducing (P < 0.05) silver leaf symptomatic zucchini plants in both trials. In Trial II, SH also delayed papaya-ring spot virus symptom development. At harvest, zucchini yield was consistently higher in SH than BG. Fruit weights and numbers of fruits varied among the subplot treatments, adding CT did not show significant improvement of zucchini yield as compared to Fert in both trials. Thus, SH proved to be a versatile strip-till cover crop for both above and below ground beneficial organism and pest management in this zucchini agroecosystem. Future work on evaluating cover crop and CT treatments need to reduce the interference from pickleworm and fruitflies damage which were both challenging pests to manage in this reduced risk pesticide system in Hawaii.

THE INVESTIGATION OF PATHOGENICITY DECREASING OF *APHELENCHOIDES BESSEY1* UNDER ARTIFI-CAIL CULTURING CONDITIONS. **Wen, Rour-Chaihn, Peichen Chen, and Tung-Tsuan Tsay.** Department of Plant Pathology, National Chung Hsing University, No.250, Kuo-Kuang Rd., South Dist., Taichung 402, Taiwan.

Four sister lines derived from one single female of *Aphelenchoides besseyi* were established to investigate the correlation between nematode culturing conditions and the pathogenicity on bird's-nest fern (*Asplenium nidus* L.). The four sister lines were reared under different conditions. The BB line was continuously cultured on bird's-nest fern for 6 generations, while the BA line was cultured on bird's-nest fern for the first 3 generations and on *Alternaria citri* PDA slant for the next 3 generations. The AA line was cultured on the *A. citri* slant for 6 generations continuously, while the AB line was cultured on

A. *citri* slant for 3 generations and switched to bird's-nest fern for the next 3 generations. At the  $3^{rd}$  and  $6^{th}$  generation, a portion of nematodes from each line were needed to inoculate bird's-nest fern and the diseased areas on the leaves were measured. The diseased areas caused by the  $3^{rd}$  generation of four sister lines did not show significant differences. However, at the  $6^{th}$  generation, BB line caused the biggest diseased area (10.87 cm<sup>2</sup>), while AA line caused the smallest diseased area (4.32 cm<sup>2</sup>). The diseased area caused by AB and BA lines did not have significant differences, they were 6.44 cm<sup>2</sup> and 5.54 cm<sup>2</sup>, respectively. Females were re-isolated from the diseased leaves, regardless of their differences in ability to cause symptoms, the numbers of their tail mucros were the same. Based on de Manian's formula of the  $6^{th}$  generation, BB line had the biggest body length and width, while the AA lines had the smallest. However, all the morphometrics were in the range of *A. besseyi* measured by Dastur in 1936. The results of our study indicated that the decreasing of bird's-nest fern pathogenicity trait might not link to the morphology traits observed in this study.

#### IN VITRO GROWTH RESPONSE OF *DACTYLELLA OVIPARASITICA* STRAIN 50 TO VARIOUS CULTURE MEDIA AND ENVIRONMENTAL FACTORS. **Witte, Hannes, J. Smith Becker, and J.O. Becker.** Department of Nematology, University of California, Riverside, CA 92521.

The nematophagous fungus *Dactylella oviparasitica* strain 50 was isolated from parasitized cysts of *Heterodera schachtii* obtained from a sugar beet cyst nematode-suppressive field site at Agricultural Operations, University of California-Riverside. The ascomycete was previously shown to be the primary agent responsible for the *H. schachtii* population suppression. The aim of this study was to determine culture characteristics and effect of environmental conditions on the growth of *D. oviparasitica* strain 50 in vitro. The strain produced dense colonies with thick aerial mycelium on enriched YPSS agar, potato dextrose agar and V-8 juice agar. Mycelial growth on low nutrient media was sparse. Growth of the fungus occurred in buffered, liquid nutrient broth at pH 4.5, 6 and 7.5. Optimum growth was observed at pH 6. No hyphal extension occurred at pH 3 or 9. The fungal growth rate was greatest at an osmotic potential of -0.3 MPa and increasing osmotic potential resulted in decreasing growth. No growth was observed at an osmotic potential of -4.3 MPa on media amended with glycerol and at -6.3 MPa on media amended with sodium chloride, potassium chloride or sucrose. The fungus grew within a temperature range from 15 to 27°C with optimum growth at 25°C. No growth was observed at 11 and 31°C. Variation of media, pH, osmotic potentials, temperatures, light regimes and use of eggs of *Meloidogyne arenaria* as a substrate did not initiate sporulation of *D. oviparasitica* strain 50.

IMPACT OF ORGANIC AMENDMENTS ON NEMATODE DISTRIBUTION WITHIN AGGREGATE FRACTIONS IN AEOLIAN SANDY SOIL. **Wu, Xia<sup>1,2</sup>, X.K. Zhang<sup>1</sup>, J. Yu<sup>1</sup>, X.M. Sun<sup>1,2</sup>, and W.J. Liang<sup>1</sup>.** <sup>1</sup>Key Laboratory of Forest and Soil Ecology, Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang 110164, China; and <sup>2</sup>Graduate University of the Chinese Academy of Sciences, Beijing 100049, China.

In order to evaluate the effect of organic amendments on soil aggregate fractions, and their interaction in soil nematode community composition and diversity, a study was initiated in aeolian sandy soil, Northeast China. Soil samples were collected from a plow layer (0-20 cm) and a plow pan (20-40 cm) in the cornfields under three different managements: 1) conventional cropping (CK, corn straw removal and no organic manure application); 2) straw retention (SR, incorporation of 9,000 kg/ha chopped corn stalk); and 3) manure application (MA, 15,000 kg/ha chicken manure input). The soil samples were fractionated into four different aggregate sizes, i.e., >2 mm (large macroaggregates), 1-2 mm (macroaggregates), 0.25-1.00 mm (small macroaggregates), and <0.25 mm (microaggregates, silt and clay fractions). The composition and diversity of soil nematode communities were determined within each aggregate fraction. The results showed that organic-amendment treatments (SR and MA) significantly increased the proportion of macroaggregates (>2 and 1-2 mm) compared to CK. The abundance of total nematodes and four trophic groups were all affected significantly by soil fractions, with higher abundance in the larger-size aggregates. Bacterivores in the <1 mm fraction and fungivores in the >2 mm fraction were significantly different among treatments, with their highest values generally in SR. The abundance of cp-2 guild was predominant at each aggregate fraction of both layers and was significantly influenced by aggregate size. The nematode channel ratio (NCR) and channel index (CI) suggested that decomposition of organic matter tended to be achieved primarily through the bacterialbased energy pathway after organic treatment application. The aggregate size determined the pore size of habitable space of soil nematodes, and was one of most important factors for soil nematode distribution. Nematode communities were limited by the degree of soil aggregation and the availability of organic resources associated with soil structure.

# OVER-EXPRESSION OF MIC3 REDUCES COTTON SUSCEPTIBILITY TO ROOT-KNOT NEMATODE. Wubben, Martin<sup>1</sup>, F.E. Callahan<sup>1</sup>, J.N. Jenkins<sup>1</sup> and J. Velten<sup>2</sup>. <sup>1</sup>USDA-ARS, 810 Highway 12 East, Mississippi State, MS 39762; and <sup>2</sup>USDA-ARS, 3810 4<sup>th</sup> Street, Lubbock, TX 79415.

While the inheritance of root-knot nematode (*Meloidogyne incognita*; RKN) resistance in cotton (*Gossypium hirsutum*) has been the focus of much research, the mechanism of the resistance at the molecular level remains largely unknown. To date, increased transcript and protein levels of *MIC3* (Meloidogyne Induced Cotton3) in galls of resistant plants remains the

only example of a gene whose expression is correlated with the onset of RKN resistance in cotton. MIC3 represents a large gene family in cotton that encode proteins approximately 14 kDa in size that lack similarity with known proteins and do not contain any known domains or motifs; furthermore, MIC3 appears to be a cotton-specific gene with no homologous sequences being identified outside the genus Gossypium. In this report, we further validate the correlation between MIC3 expression and RKN resistance via overexpression of *MIC3* in the RKN-susceptible line Coker312. A *MIC3* overexpression cassette driven by the CaMV35S promoter was constructed using the binary vector pBI121. Transgenic cotton lines harboring this cassette were created using Agrobacterium tumefaciens. Five (5) homozygous T2 lines were identified that showed elevated MIC3 transcript and protein levels in roots and leaves compared to non-transgenic controls. For the RKN assays, data from two independent experiments showed that both high and low levels of MIC3 overexpression affected RKN egg production but not RKN-induced root galling. We found that the transgenic line 11-1-1Top, which showed 14.7-fold higher MIC3 transcript in uninfected roots compared to the non-transgenic control, supported 70% fewer RKN eggs/plant compared to the susceptible control Coker312. In contrast, the transgenic line 14-11-1Top, which showed the lowest level of MIC3 overexpression of the five homozygous lines, reduced RKN eggs/plant by only 35% compared to Coker312. None of the transgenic lines showed gall index scores that were significantly different from the susceptible controls. In contrast to RKN, MIC3 overexpression did not affect cotton susceptibility to neither the reniform nematode (Rotylenchulus reniformis) nor the foliar pathogen Heliothis zea.

POPULATION DEVELOPMENT OF *ROTYLENCHULUS RENIFORMIS* IN DIFFERENT SOIL TEXTURES WITHIN A COMMERCE SILT LOAM FIELD. Xavier, Déborah<sup>1</sup>, C. Overstreet<sup>1</sup>, E.C. McGawley<sup>1</sup>, M.T. Kularathna<sup>1</sup>, D. Burns<sup>2</sup>, R.L. Frazier<sup>3</sup>, and C.M. Martin<sup>1</sup>. <sup>1</sup>302 Life Sciences Building, Plant Pathology Dept., Baton Rouge, LA 70803; <sup>2</sup>P.O. Box 438, St. Joseph, LA 71366; and <sup>3</sup>114 N Cedar, Tallulah, LA 71282.

The effect of soil texture on *Rotylenchulus reniformis* population development was evaluated in two Commerce silt loam fields. In this study, two commercial cotton fields located in Northeast Louisiana were used with both fields having highly variable soil texture and *R. reniformis* as the dominant nematode present. The fields were divided into five different zones based on apparent electrical conductivity (EC) data from the Veris 3100 EC<sub>a</sub> soil mapping implement. Treatments consisted of treated (1,3-dichloropropene at 28.1 l/ha) and untreated rows (12 rows wide) running through the entire length of each field with four replications. Nematode populations at planting and after harvest and soil texture were estimated from sample sites collected in each zone. Additionally, a single core (15.2 cm diameter) at each of the sampling sites was taken at planting in 15.2 cm increments until 61 cm and assessed for nematode populations and soil texture. All the sample sites were identified in the field by using a Trimble Juno handheld GPS receiver and a FarmWorks SiteMate Pro program. No significant differences in nematode populations were found between treated and untreated treatments in either field. Across the fields, sample sites with clay contents of 10-20% had higher nematode populations. In both fields, the highest populations of *R. reniformis* were found in the lower depths (30.5 to 61 cm). Populations of the nematode were negatively correlated with clay content in field one (R<sup>2</sup>=-0.61; P=0.000) and field two (R<sup>2</sup>=-0.32; P=0.08). Nematode populations by zones followed the same trend for these two fields. The use of Veris 3100 EC<sub>a</sub> soil mapping implement may be a good option to define zones within a field, aiding in the nematode management.

#### FIELD EVALUATION OF *STREPTOMYCES RUBROGRISEUS* HDZ-9-47 FOR BIOLOGICAL CONTROL OF ROOT-KNOT NEMATODE *MELOIDOGYNE INCOGNITA*. Xue, Hui, Na Jin, Xueyan Wang, Wenjing Li, and Heng Jian. Department of Plant Pathology, China Agricultural University, Beijing 100193, China.

Root-knot nematode (RKN) is one of the most economically important genera of plant-parasitic nematodes on field crops in China. Biocontrol as an alternative against RKN has been broadly studied and applied. Streptomycetes spp. is widely distributed in soil, and some species have biological control potential against RKN. Streptomyces rubrogriseus HDZ-9-47 was originally isolated from the egg mass of *Meloidogyne hapla*. Preliminary tests indicated that S. rubrogriseus could reduce egg hatching rate of M. incognita by 63% and its 4% ferment filtrate led to 84% mortality of J2 in vitro. In 2008 – 2011, HDZ-9-47 was evaluated to control M. incognita on tomato in field at Tongzhou District, Beijing, China. Control efficacies of the filtrate containing various spore concentrations of HDZ-9-47 ( $10^9$ ,  $10^{10}$ ,  $10^{11}$  and  $10^{12}$  spores per plant) were compared at 90 days after transplant. Each treatment consisted of one row, 15 plants, 4 replications, which was laid out in a randomized complete design. Compared to the untreated control, application with 10<sup>12</sup> spores of HDZ-7-24 per plant reduced the galling indices by 52-70%. In another test, combination of bio-fumigation soil treatment using cabbage residues followed by application of  $10^{12}$  spores of HDZ-9-47 reduced the galling indices by 81-88%. It was higher than those of HDZ-9-47 (52-70%) or bio-fumigation (46-58%) alone, and was not significantly different from the fosthiazate (68-80%). Furthermore, HDZ-9-47 against *M. incognita* on cucumber was evaluated for three successive growing seasons from 2010 to 2011. The results showed that combination of bio-fumigation soil treatment using cabbage residues and application of  $10^{12}$  spores of HDZ-9-47 per plant reduced the galling indices by 48-53% at 90 days after transplant, which was equal to that of fosthiazate (50%). Our results demonstrated that soil pre-treatment with bio-fumigation followed by drench application of ferment filtrate of HDZ-9-47 had synergistic effects against RKN in field. S. rubrogriseus HDZ-9-47 was a promising agent for biological control of RKN and future researches should focus on the identification of nematicidal compounds, optimization of fermentation technology and commercial formulation of *S. rubrogriseus*.

MOLECULAR CHARACTERIZATION OF *MELOIDOGYNE INCOGNITA* FROM A PEACH ORCHARD IN NORTH CAROLINA. **Ye, Weimin<sup>1</sup>, David Dycus<sup>1</sup>, and Steve Koenning<sup>2</sup>.** <sup>1</sup>Nematode Assay Section, Agronomic Division, North Carolina Department of Agriculture & Consumer Services, Raleigh, NC 27607; and <sup>2</sup>Department of Plant Pathology, North Carolina State University, Raleigh, NC 27695.

A 2012 survey of root-knot nematodes in a peach orchard in Moore County, NC, revealed severe root-knot nematode infection in some rows of trees of unknown rootstock. Trees with Guardian rootstock, which protects trees from peach tree short life disease, were healthy. Second-stage juveniles in samples taken around infected trees numbered 3,590 per 500 cc soil. The species was identified as *Meloidogyne incognita* through PCR and DNA sequencing on the near-full-length small subunit rDNA gene, D2/D3 expansion segments of the large subunit rDNA gene, internal transcribed spacer, intergeneric spacer, mitochondrial DNA cytochrome oxidase subunit II and the 16S rRNA gene, histone gene and an uncharacterized protein coding gene. Species-specific molecular primers and probes were designed to identify *M. incognita* based on the histone gene and were applied in real-time PCR assay for rapid species identification.

OCCURRENCE OF *PASTEURIA* SPP. ATTACKING VARIOUS NEMATODES IN PASTURES OF BINGOL, TURKEY. **Yildiz, Senol<sup>1</sup> and Z. A. Handoo<sup>2</sup>.** <sup>1</sup>Department of Horticulture, Michigan State University, East Lansing, MI, 48824; and <sup>2</sup>USDA-ARS Nematology Laboratory, Beltsville, MD, 20705.

In spring 2011, a survey of plant-parasitic nematodes in pastures was conducted in Bingol Province, a highly mountainous and temperate area in Eastern Anatolia. The province relies on pastures to support its livestock industry, which is the most important driving force of the economy. A total of 24 samples were collected from four districts (Bingol, Genc, Karliova and Solhan) in the province. Nematodes were extracted and fixed in TAF, mounted on permanent slides, and identified to species. In three samples (8%), *Helicotylenchus platyurus*, *Pratylenchus thornei* and *Tylenchorhynchus brassicae* were found heavily attacked by *Pasteuria* endospores; endospore density ranged between 3-15 per individual nematode. The mean diameter of the endospores measured 4.5 micrometers with a standard deviation of 0.74 micrometers. Photomicrographs were taken with a digital camera attached to a compound microscope. This study represents the first report of *Pasteuria* spp. from an uncultivated habitat in Turkey and the first report for the presence of *Pasteuria* in the Eastern Anatolia region. *Pasteuria* may be a potential biological control agent of plant-parasitic nematodes in pastoral areas where no control practices are applied; however, further research on its spatial distribution is needed to evaluate its potential.

COLLABORATIVE EFFORTS TOWARDS UNDERSTANDING THE BIOLOGICAL STRUCTURES AND FUNC-TIONS OF SELECTED SOIL GROUPS IN RURAL GHANA, MALAWI AND KENYA. Yildiz, Senol<sup>1</sup>, T. Teal<sup>2</sup>, **R. Mkandawire<sup>1,6</sup>**, **J. van Ravansway<sup>3</sup>**, **A. Thuo<sup>4</sup>**, **C. Kwoseh<sup>5</sup>**, **T. Adjei-Gyapong<sup>5</sup>**, **V. Saka<sup>6</sup>**, **M. Lowole<sup>6</sup>**, **G.N. Karuku<sup>4</sup>**, **P.M. Wachira<sup>4</sup>**, **V.N. Gathaara<sup>7</sup>**, **J.W. Kimenju<sup>4</sup>**, **J. Qi<sup>3</sup>**, **T. Schmidt<sup>2</sup>**, and **H. Melakeberhan<sup>1</sup>**. <sup>1</sup>Agricultural Nematology Lab, Dept. of Horticulture, <sup>2</sup>Microbiology and Molecular Genetics and <sup>3</sup>Center for Global Change and Earth Observation, Michigan State University, East Lansing, MI 48824; <sup>4</sup>University of Nairobi, Kenya; <sup>5</sup>Kwame Nkrumah University of Science and Technology, Ghana; <sup>6</sup>University of Malawi, Malawi; and <sup>7</sup>Kenya Agricultural Research Institute, Nairobi.

Degrading health of sub-Saharan African soils is a major impediment to the Millennium Development Goal and the strategy of poverty reduction for Africa through the Comprehensive Africa Agricultural Development Program. Without addressing soil degradations, vital ecosystem services are unlikely to return to nor remain at levels that can sustain viable human populations. Improving ecosystem services and reducing poverty require integrated understanding of the connections among terrestrial agro-ecosystem degradation, habitat and biodiversity loss, lower agricultural yield, food insecurity, and forced population migration. This Howard G. Buffett Foundation-funded and collaborative project among MSU, KNUST, UoN, UoM, and the New Partnership for African Development (NEPAD) Agency is a foundation towards developing scalable soil health management strategy in soil groups. Using nematode assemblage and total microbial analyses as major soil ecosystem change indicators, the objective in this phase of the project is to establish baseline information on biological structure and function of Ferralsols, Lithosols and Nitosols in different regions and production practices of Ghana, Malawi and Kenya. Over 500 soil samples have been collected from disturbed (agricultural) and undisturbed (natural) ecosystems during March and April of 2012. As part of accounting for the role of anthropogenic factors on land use practices, cropping history, land ownership, socioeconomic, and related cultural information were also considered. Field observations suggest large differences in land use practices within and across regions. Analyses of preliminary data will be presented.

THE CANADIAN NATIONAL COLLECTION OF NEMATODES: AN OVERVIEW. **Yu, Qing.** Curator for the Canadian National Collection of Nematodes, Ottawa, ON Canada.

The Canadian National Collection of Nematodes is one unit of the Canadian National Collection of Insects, Arachnids, and Nematodes. Since 1917, Agriculture and Agri-Food Canada has been the custodian of the collection, which is currently

housed on the Central Experimental Farm in Ottawa, ON. The nematode collection was created by Dr. A. Baker in 1945, with the primary mandate of discovering and documenting the Canadian nematode fauna, although the collection also houses significant international depositions. The collection was expanded by R. Mulvey and R. Anderson, the curators following Dr. Baker. Significant contributions were also made by the associated taxonomists Drs. L-Y. Wu and B. Ebsary. Other Canadian nematologists have deposited their specimens in the collection over the years, including Drs. W. B. Mountain, J. Webster, T. Vrain, J. Townshend, T. Oltholf and J. Potter. The collection consists of the type collection, the general collection and the literature collection. The type collection has over 500 species of primary and secondary types, and the general collection has over 10,000 accessions. The Canadian National Collection of Nematodes is the only one of its kind in Canada. Since its creation, it has not only successfully served in supporting research and regulatory activities in Canada, but it has become a major repository of nematode specimens from nematologists around the world. Efforts are underway to digitize the collection.

### *DITYLENCHUS DESTRUCTOR* Thorne, 1945 (TYLENCHIDA: ANGUINIDAE) IN CANADA. Yu, Qing. Eastern Cereal and Oilseed Research Center, Agriculture and Agri-Food Canada, Ottawa, ON Canada.

The potato rot nematode, *Ditylenchus destructor* Thorne 1945, is a serious pest in a number of crops, and it is internationally quarantined. In Canada, its distribution is reported to be limited. Prior to the recent find in garlic in Ontario in 2011, *D. destructor* had been reported in potatoes in Prince Edward Island (PEI) in 1946, and in potatoes (1953) and Iris (1961) on Vancouver Island, British Columbia (BC), although these latter findings have not been recognized. Re-examination of the BC and PEI findings and comparison to the recent finding in Ontario was possible because the nematode specimens from these earlier studies were deposited in the Canadian National Collection of Nematodes. Morphologically, the main characters of these nematodes match those of *D. destructor*. Some morphometric differences were observed among the nematodes of the 4 isolates. We present these data to eliminate any possible confusion about the identity of the *D. destructor* in Canada.

USING MITOGENOMIC AND NUCLEAR RIBOSOMAL SEQUENCE DATA TO INVESTIGATE THE PHYLOGENY OF *XIPHINEMA AMERICANUM* POPULATIONS FROM THE UNITED STATES. **Zasada, Inga<sup>1</sup>, A. Peetz<sup>1</sup>, A. Smythe<sup>2</sup>, D. Howe<sup>3</sup>, D. Cheam<sup>3</sup>, and D. Denver<sup>3</sup>.** <sup>1</sup>USDA-ARS Horticultural Crops Research Laboratory, 3420 NW Orchard Ave., Corvallis, OR 97330; <sup>2</sup>Department of Biology, Hamilton College, Clinton, NY 13323; and <sup>3</sup>Department of Zoology, Oregon State University, Corvallis, OR 97330.

Species within the Xiphinema americanum-group are considered economically important because they vector nepoviruses which can cause considerable damage to a variety of agricultural crops. The taxonomy of the X. americanum-group is historically controversial, with the number of putative species being the subject of debate. Some members of the X. americanum-group have been linked with virus transmission while others have not. Continued phylogenetic analysis of this group is highly desirable as it may ultimately reveal genetic differences between species which do vector nepoviruses and those which do not. *Xiphinema americanum* populations were collected from 12 geographically disparate locations across the U.S. representing different crops and presence/absence of nepoviruses. DNA was extracted from at least three individuals from each population. A portion of the 18S nuclear ribosomal DNA (rDNA) was sequenced for all isolates, and mitochondrial genomes were sequenced for numerous isolates in parallel using high-throughput DNA sequencing technology. The internal transcribed spacer region 1 (ITS1) of rDNA was cloned and three clones per individual were sequenced. Sequences were subjected to phylogenetic analysis and compared. Analysis of the 18S rDNA revealed virtually identical sequences across all populations (only one polymorphic site). Conversely, analysis of the mtDNA sequence data indicated the presence of three separate and highly divergent (up to  $\sim 20\%$  pairwise divergence) lineages of X. americanum within the 12 populations; these lineages did not correlate with geographic location, host, or ability to transmit virus. ITS1 sequence data suggested two major lineages which were generally geographically distinct with eastern (Ohio, Pennsylvania, North Carolina) and western (Washington, Colorado, Oregon) lineages of X. americanum; an exception was a population from New York that grouped with the western lineage. In addition, ITS clones revealed that most populations contained several paralogous sequences that failed to form clades with other sequences from the same population. The inherent heterogeneity in ITS1 sequence data within an individual and population and lack of informative sites in 18S rDNA analysis suggests that mitogenomics may be more informative in sorting out the taxonomic confusion of the X. americanum-group.

RESPONSE OF RED RASPBERRY (*RUBUS IDAEUS*) VARIETIES TO *PRATYLENCHUS PENETRANS*. Zasada, Inga A.<sup>1</sup>, and T.W. Walters<sup>2</sup>. <sup>1</sup>USDA-ARS Horticultural Crops Research Laboratory, 3420 NW Orchard Ave., Corvallis, OR 97330; and <sup>2</sup>Washington State University Northwest Research and Education Center, Mt. Vernon, WA 98273.

Washington State is the nation's largest producer of red raspberries (*Rubus idaeus*) for processing. *Pratylenchus penetrans* is a major constraint to the industry, shortening the productive lifetime of many plantings. To improve the management of this plant-parasitic nematode, information on the impact of this nematode on the establishment and productivity of raspberry would be beneficial. A field trial was established to evaluate the response of eight red raspberry varieties (Anne, Caroline, Cascade Bounty, Chemainus, Heritage, Meeker, Saanich, and Willamette) as well as *R. niveus* and *R. leucodermis* to

P. penetrans. Rubus niveus and R. leucodermis selections were included in the experiment because they were identified as being resistant to P. penetrans in greenhouse evaluations. The experiment was a split plot design with fumigated (1,3-dichlropropene and chloropicrin) or non-fumigated main plots and plant genotype as subplots. Non-fumigated main plots had an average of 124 P. penetrans/100 g soil at planting while few nematodes were detected in fumigated main plots. Six months after planting one plant was removed from each plot and shoot and root biomass determined as well as number of P. penetrans/g root. Pratylenchus penetrans reduced shoot biomass of Meeker, Saanich, Willamette, Chemainus, Cascade Bounty, and Anne grown in non-fumigated soil by at least 24% compared to those grown in fumigated soil. Root biomass of all of the red raspberry varieties evaluated, as well as R. niveus and R. leucodermis was reduced by at least 22% when grown in soil infested with P. penetrans compared to plants grown in fumigated soil. After six months, P. penetrans populations increased dramatically on all varieties with the extremes being 5,281 P. penetrans/g root recovered from Anne and 765 *P. penetrans*/g root recovered from *R. niveus*. There was a significant difference (P < 0.05) between the number of P. penetrans/g root recovered from R. niveus compared to Anne, Cascade Bounty, Caroline, and Saanich. Regardless of red raspberry variety, P. penetrans has the potential to significantly reduce plant growth during establishment. While R. niveus and R. leucodermis supported the smallest increase in root populations of P. penetrans, it is apparent that under heavy P. penetrans pressure these Rubus species are not fully resistant to P. penetrans. These results clearly demonstrate the need for pre-plant management of *P. penetrans* in fields where red raspberry will be planted.

NEW *DIPLOSCAPTER* SP. (RHABDITIDA: DIPLOSCAPTERIDAE) FROM THE NATIVE ANT, *PROLASIUS ADVENUS*, IN NEW ZEALAND. **Zhao, Zeng Qi<sup>1</sup>, K.A. Davies<sup>2</sup>, E.C. Brenton-Rule<sup>3</sup>, J. Grangier<sup>3</sup>, M.A.M. Gruber<sup>3</sup>, R.M. Giblin-Davis<sup>4</sup>, and Philip J. Lester<sup>3</sup>. <sup>1</sup>Landcare Research, Private Bag 92170, Auckland Mail Centre, Auckland 1142, New Zealand; <sup>2</sup>Australian Centre for Evolutionary Biology and Biodiversity, School of Agriculture, Food and Wine, University of Adelaide, Waite Campus, PMB 1, Glen Osmond, SA 5064, Australia; <sup>3</sup>Centre for Biodiversity and Restoration Ecology, Victoria University of Wellington, PO Box 600, Wellington 6140, New Zealand; and <sup>4</sup>University of Florida-IFAS, Fort Lauderdale Research and Education Center, 3205 College Ave., Davie, FL 33314.** 

A newly-discovered species of *Diploscapter* sp. was recovered from dissections of the ant *Prolasius advenus* and from its nests from beech (Nothofagus) forests of the northern South Island and the southern North Island in New Zealand. Both the ant and its associated *Diploscapter* sp. appear to be native to New Zealand. This is a new host record for *Diploscapter* and the first report of an ant associate from the southern hemisphere. Second-stage juveniles (J2) (based upon size of the gonad primordium) and J3 and J4s were extracted from ant heads and free-living J3 and J4 juveniles were collected from nest detritus, but no dauer juveniles were observed. Associative juveniles of *Diplogaster* sp. were observed nictating, behaviour suggestive of host-seeking. Males were not recovered from ant nests or from cultured nematodes corroborating previous reports that they are rare or absent in this genus. Adult females were observed with bilateral symmetry of the head, characteristic dorsal and ventral projections of the putative cheilostom with paired hook-like structures or hamuli, expansive membranous lateral lip flaps or laciniae; gymnostom and stegostom with parallel walls; a swollen procorpus, large terminal bulb with a strong valve; paired ovaries with medial vulva; and a short conoid tail with slender pointed or spicate tip. Scanning electron micrographs of the head confirmed that the lateral laciniae with finger-like tines or filopodia are moveable (alternately covering and exposing the mouth). These lateral lip flaps originate posterior to the stoma, but anterior to the porelike amphidial openings. The anterior margin of the cheilostom possesses apomorphic lateral bell-shaped projections and the hamuli are broader and less pointed than other species that have been examined. Molecular phylogeny of near full length small subunit (SSU), D2/D3 expansion segments of the large subunit (LSU) rRNA gene, and heat shock protein 90 (Hsp90) gene showed that this *Diploscapter* sp. is monophyletic with *Diploscapter* species and isolates available in GenBank, but is on an independent trajectory supporting separate species status.

NEMATODE BEHAVIOR IN RELATION TO GRAPE ROOTS IN DUAL CULTURE. **Zheng, Liang<sup>1</sup>, H. Ferris<sup>1</sup>, and M.A. Walker<sup>2</sup>.** <sup>1</sup>Department of Entomology and Nematology, University of California, Davis, CA 95616; and <sup>2</sup>Department of Viticulture and Enology, University of California, Davis, CA 95616.

We examined various techniques for dual culture of grapes and nematodes, including different methods of surfacesterilizing nematodes and plant tissues, different media concentrations and rates of hormone amendment. We also compared culture of tissues derived from plant nodes and petioles, and incubation under conditions of light and dark. We monitored the behavior and productivity of *Pratylenchus vulnus* and *Meloidogyne incognita* in relation to grape rootstock selections with differing levels of resistance. Roots emerging from stem nodes developed more rapidly than those from petioles. In media amended with  $0.54\mu$ M/µL NAA ( $\alpha$ -Naphthaleneacetic Acid), bud development occurred 2-5 days earlier, and roots were double the size of those in unamended controls. There were no observable negative effects of hormone concentration on the nematodes. Culture in light promoted plant growth and longevity of the dual culture system; in the dark, leaves lost turgor and roots died. Under such conditions, the migratory endoparasites *P. vulnus* root tissues while eggs of *M. incognita* were vacuolated and many were dead. Differing concentrations of salts in MS (Murashige & Skoog Basal Medium with vitamins), SM (Shoot Medium plus NAA), and NN (Nitsch & Nitsch Basal Medium with vitamins) had a little effect on survival of

#### 502 Journal of Nematology, Volume 44, No. 4, December 2012

*P. vulnus* or *M. incognita*. Plant growth rate differed with agar concentrations with bud development slower at concentrations > 5g/L. At concentrations >8g/L some nematodes had difficulty penetrating the media and eventually died. Most *P. vulnus* and *M. incognita* J2s entered tissues behind the root tip within 24 hours. Throughout the observation period there were always some *P. vulnus* individuals outside the root, either because they never entered or because they had entered and then exited. After about 2 months, large numbers of *P. vulnus* vacated the deteriorating roots. The rate of exit of *P. vulnus* from roots was greater from roots resistant to *Meloidogyne* and *Xiphinema* than from roots of susceptible cultivars. Fewer *Meloidogyne* J2 entered roots of resistant UCD-GRN1 than susceptible cv Colombard within the same time period. While the nematodes developed and produced eggs on cv Colombard, none developed beyond the J3 stage on the resistant genotype.

IDENTIFICATION OF *HETERODERA* SPECIES IN CHINA'S TROPICAL AND SUBTROPICAL AREAS. Zhuo, Kan, H.H. Wang, H.L. Zhang, and J.L. Liao. Laboratory of Plant Nematology, South China Agricultural University, Guangzhou, China 510642.

*Heterodera* is a group of important plant pathogens, but little work on *Heterodera* has been conducted in China's tropical and subtropical areas. During the past three years, surveys for the presence of *Heterodera* species in China's tropical and subtropical areas have been performed. Three new species including one species from Hainan Province and two species from Guangdong Province were under description by using comparative morphological, morphometric and molecular approaches. The phylogenetic trees based on D2D3 of the 28S rDNA and rDNA-ITS showed that the new species from Hainan belongs to the '*Afenestrata*' group, while two new species belong to the '*Cyperi*' group. In addition, *H. koreana* from Jiangxi Province, *H. graminophila* from Guangdong Province and *H. elachista* from Guangxi Province were also identified. *H. koreana* and *H. graminophila* are new records in China.