

ABSTRACTS

ASSESSMENT OF RHIZOBACTERIAL STRAINS ON THE CONTROL OF THE GOLDEN CYST NEMATODE, *GLOBODERA ROSTOCHIENSIS* IN CHILE. **Aballay, E.¹, C. Flores¹, and S. Prodan¹.** ¹Departamento de Sanidad Vegetal, Universidad de Chile, P.O. Box 1004, Santiago, Chile.

Potato is one of the four most relevant annual crops for Chilean agriculture, with more than 50,000 ha cultivated within the country. Several pests and diseases affect the crop, with the plant parasitic nematode *Globodera rostochiensis* one of the most frequent, affecting its growth and productivity in many areas. This pest has been found in new areas during the last three years. Farmers try to decrease damage through crop rotations and management activities, but the main tool is the use of chemical nematicides, organophosphates and carbamates applied at sowing. The use of bioantagonists has not been evaluated under experimental or practical conditions, so the aim of this work was to evaluate some rhizobacteria isolated from grapevines roots for the control of this nematode under potted plant conditions. Four strains were cultivated in TSB at 22 °C, *Bacillus brevis* 37, *B. weihenstephanensis* 25, *Oerskovia turbata* 55 and *Pseudomonas putida* 1301, to get a final concentration of 1×10^6 UFC mL⁻¹. The susceptible potato cultivar used was Desiree. Small tubers were dipped for 5 minutes in bacterial suspension, planted in a 5 L pot with naturally infested soil and kept in an open room under ambient environmental conditions during the summer. Two assays were performed using the same kind of soil, one (assay 1) with a density of 76 and the other one (assay 2) with 22 cysts per 250 cm³ of soil. Each experiment included five treatments, four rhizobacteria and a control with untreated soil, for a total of ten replicates per treatment. The mean number of live eggs was 180 per cyst. After four months growth, plants were uprooted and foliage and roots fresh weight were measured. Second stage juveniles and cysts per 250 cm³ soil were also determined. For assay 1, no significant differences were detected for nematode populations between treatments. The same results were shown for foliage weight, but for roots the strain 55 had a greater weight than the other strains and the control ($p < 0.05$). For assay 2, with lower initial populations, the strain 55 was different from the control with respect to the number of cysts per soil unit. Some differences were detected for plant parameters. According to these results, the strain 55 of *Oerskovia turbata* shows good potential for continued assessment under different soil and weather conditions.

BIOSYNTHESIS OF SILVER NANOPARTICLES (AgNPs) BY *PSEUDOMONAS AERUGINOSA* AND THEIR POTENTIAL AS NEMATICIDAL ACTIVITY. **Abdelmoneim, T.^{1,2} and S.I. Massoud².** ¹Biology Department, Faculty of Science, University of Jeddah, Jeddah, Saudi Arabia. ²Suez Canal University, Faculty of Agriculture, Department of Agricultural Botany, Ismailia, Egypt.

The gram negative bacterium, *Pseudomonas aeruginosa* was isolated from agricultural wastewater in Ismailia region, Egypt. This specie was used for biosynthesis of silver nanoparticles (AgNPs) as a potential nematicidal agent via reduction of silver nitrate (AgNO₃) in cell free protein from *P. aeruginosa*. The AgNPs were confirmed by transmission electron microscopy (TEM), analysing surface plasmon resonance using UV- visible spectrophotometer, and energy dispersive X-ray spectroscopy (EDX). The results showed that the size of AgNPs, formed by *P. aeruginosa* ranged from 18.91 to 23.45 nm. Second stage juveniles (J2) of root-knot nematode *Meloidogyne* sp. were exposed to either 0, 100, 150, 200, or 250 µg/ml of AgNPs in 5ml of nematode suspension (500 J₂/ml) for 40 min before they were used to infect tomato seedlings. The best results were shown at 200 and 250 µg/ml of AgNPs, with decreases in the numbers of J2 in soil (40, 56%) and inside tomato roots (30, 44%) as well as the gall numbers (46, 68%) and egg masses/g of root (50, 76%) as compared with control. Biosynthesis of the AgNPs is ecofriendly, as it is free from any solvent or toxic chemicals, is easily adjustable to large scale production, and provides a useful method for manufacturing of the biosynthetic product.

DIVERSITY OF RHABDITID NEMATODES (NEMATODA: RHABDITIDA) IN XERIC ENVIROMENTS FROM SOUTHERN IBERIAN PENINSULA: THE CASE OF RIA FORMOSA-DOÑANA AREA. **Abolafia, J.¹, A.C. Silva^{1,2}, Y. Martínez-Hervás¹, R. Peña-Santiago¹.** Departamento de Biología Animal, Biología Vegetal y Ecología, Universidad de Jaén, Campus “Las Lagunillas” s/n, 23071-Jaén, Spain. ²Departamento de Ciencias Ambientales, Universidad de Castilla-La Mancha. Campus Tecnológico de la Fábrica de Armas, Avenida de Carlos III s/n. 45071-Toledo, Spain.

Nematodes are highly diverse organisms. Some of them, especially the representatives of the order Rhabditida, are able to dwell in extreme habitats such as xeric soils lacking humidity. These environments are inhospitable areas where the species develop morphological and functional adaptations to become drought resistant and to survive. Xeric soils are known to occur in several areas of southern Iberian Peninsula. This is the case for the coastline sand dunes of the Cadiz Gulf, between Ria Formosa Natural Park (Portugal) and Doñana National Park (Spain), where three separate locations were sampled: Manta Rota, Islantilla, and Matalascañas. The study of their nematode fauna revealed the existence of a rich community of rhabditid

forms consisting of 16 species, 11 genera (*Acrobeles*, *Acrobeloides*, *Cephalobus*, *Chiloplacus*, *Dolichorhabditis*, *Eucephalobus*, *Heterocephalobellus*, *Nothacrobeles*, *Stegelleta*, *Panagrolaimus* and *Pseudacrobeles*) and three families (Cephalobidae, Panagrolaimidae and Peloderidae). Four species (*Chiloplacus magnus*, *Heterocephalobellus magnificus*, *Nothacrobeles lanceolatus* and *Stegelleta incisa*) are tentatively characteristic of xeric environments. One of them, *N. lanceolatus*, is a rare taxon, only reported (endemism?) hitherto from this region. Illustrations of these species are provided in order to show their more relevant diagnostic characters.

ADAPTATION AND EXAPTATION: NEMATODES PROBABLY DON'T LIVE ON MARS (BUT THEY COULD IF THEY WANTED TO). Adams, B.¹, B.N. Adhikari², X. Xue¹, and D.H. Wall³. ¹Department of Biology and Evolutionary Ecology Laboratories, Brigham Young University, Provo UT 84602, ²USDA-ARS, Tucson, AZ 85721, ³Department of Biology and Natural Resource Ecology Laboratory, Colorado State University, Fort Collins, CO 80523.

Nematodes that inhabit the Antarctic dry valleys must cope with multiple environmental stresses, including high (and dry) winds, rapid desiccation, and extreme variations in temperature and water availability. A handful of nematode species not only persist, but also seemingly thrive in these harsh environments yet we know little of their ecological amplitude or mechanisms by which they can survive multiple, extreme forms of environmental stress. To reveal the molecular genetic mechanisms of freezing and anhydrobiotic survival, we explored patterns of gene expression in the desiccation and freeze tolerant Antarctic nematode, *Plectus murrayi* during different types and stages of stress. Interestingly, heat shock and antifreeze proteins are constitutively expressed under normal conditions, but are down-regulated under desiccation stress. The adaptive responses to freezing and desiccation appear to be coupled; temporal analyses of gene expression show that acclimation to mild stress promotes survival of harsher stress. Putative adaptations to desiccation stress promote enhanced cold tolerance, and slow dehydration enhances the freeze tolerance response. As anhydrobiosis and tolerance to freezing is broadly distributed among taxa elsewhere in the world, we question which of these traits that define their current ecological amplitudes are the result of selection pressures imposed by their current environment, and those which are co-opted from traits that evolved under different environmental conditions, perhaps elsewhere in the universe.

IDENTIFICATION OF SOUTH AFRICAN MELOIDOGYNE SPECIES AND THEIR AGGRESSIVENESS ON TOMATO. Agenbag, M.¹, H. Fourie¹, C.M. Mienie¹, M. Marais², M. Daneel³, and G. Karssen⁴. ¹North-West University, Unit for Environmental Sciences and Management, Private Bag X6001, Potchefstroom 2520, South Africa, ²Agricultural Research Council-Plant Protection Research Institute, ³Nematology Unit, Biosystematics Division, Private Bag X134, Queenswood 0121, South Africa, ⁴Agricultural Research Council – Institute for Tropical and Subtropical Crops, Private Bag X11208, Nelspruit, 1200, South Africa, ⁴National Plant Protection Organization, PO Box 9102, Wageningen, The Netherlands 6700 HC.

Root-knot nematodes (*Meloidogyne* spp.) globally parasitize a wide range of crops and hence impact adversely on yield and quality. Except for the four economically most important root-knot nematode pests (*Meloidogyne arenaria*, *M. hapla*, *M. incognita* and *M. javanica*) that parasitize crops in South Africa, *M. enterolobii* (= *M. mayaguensis*) has also been associated with guava, green pepper, potato and tomato locally. The aims of the study were to i) identify *Meloidogyne* spp. from diagnostic and research samples (28 in total) using morphological and molecular approaches and ii) determine the pathogenicity of 11 selected *Meloidogyne* populations in a greenhouse trial. The SCAR - PCR method was used for molecular analyses, while various morphological characteristics of mature females were also recorded. Approximately 1 000 eggs and second-stage juveniles (J2) of the selected *Meloidogyne* populations were inoculated on roots of a susceptible tomato cultivar (Rodade) for the pathogenicity study. *Meloidogyne arenaria*, *M. incognita* and *M. javanica* as well as *M. enterolobii* were identified by means of molecular and morphological identifications. These species occurred either as monoculture or mixed populations. Aggressiveness of the 11 selected *Meloidogyne* populations differed substantially within and among species. The most aggressive population with the highest Rf (203) was represented by a monoculture *M. javanica* population (obtained from potato roots), while a monoculture *M. enterolobii* population (isolated from guava roots) were the least aggressive (Rf = 18). The 2nd, 3rd and 4th most aggressive populations constituted of mixed populations that contained combinations of *M. enterolobii*, *M. incognita* and *M. javanica*. Identification of *M. enterolobii* will contribute towards research aimed at determining the distribution, life cycle and pathogenicity of this emerging pest. Knowledge obtained on the aggressiveness of *Meloidogyne* populations also adds valuable and useful information that researchers and farmers can use to carefully plan and construct management strategies to combat these pests in local crop production systems.

INCIDENCIA DE NEMATODOS FITOPARÁSITOS EN EL CULTIVO DE MAÍZ (*Zea mays* L.) EN LA REGIÓN DE PUNO- PERU. Aguilar-Gomez, M.¹, L.M. Israel¹, B.P. Rosario¹, M.C. Zheyila¹, Z.T. Noely¹, C.J. Shadam¹, G.A. Sthewart¹, and F.Ch. Yeni¹. ¹Universidad Nacional del Altiplano, Puno, PE.

El maíz es un cereal de importancia mundial por sus diferentes usos industriales y valor nutritivo. Los efectos ocasionados por la presencia de nematodos en el cultivo de maíz dependen del tipo de control y manejo utilizado por el agricultor. El objetivo del presente estudio es evaluar la incidencia de nematodos fitoparásitos asociados al maíz en la región de Puno - Perú.

Se recolectó 121 muestras de suelo en los distritos de Chupa, Sandía, San Juan del Oro, Cuyocuyo, Ayapata, Ollachea y San Gabán. Se procesaron por el método de fluctuación centrífuga en solución sacarosa. Los géneros identificados de nematodos fitoparásitos fueron *Helicotylenchus* spp., *Mesocriconema* spp., *Globodera* spp., *Xiphinema* spp., *Rotylenchus* spp. *Dorylaimus* spp. y nematodos de vida libre (58.68; 59.50; 49.58; 18.18; 19.83; 0.82 y 100% respectivamente). Es necesario saber identificar los síntomas que pueden ocasionar este tipo de organismos dañinos para no confundirlos con plagas o enfermedades y así poder realizar un adecuado manejo.

RESPONSE OF SEVEN ELITE CASSAVA (*MANIHOT ESCULENTA* CRANTZ) VARIETIES TO INFECTION BY *MELOIDOGYNE INCOGNITA* AND OTHER NEMATODES. **Akinsanya, K.¹ and S.O. Afolami¹.** ¹ Department of Crop Protection, Federal University of Agriculture, P.M.B. 2240, Abeokuta, Ogun State, Nigeria. (¹Current Address: Augustine University, P.M.B. 1010, Ilara-Epe 106101, Lagos State, Nigeria).

Cassava (*Manihot esculenta*) has seldom received attention from nematologists because of the erroneous belief that it was too hardy to be significantly damaged by nematodes. In this study, seven cassava varieties were evaluated for their response to infection by *Meloidogyne incognita* in a pot experiment and a mixture of ten other nematodes under field conditions. The 7x2 factorial pot experiment was comprised of seven cassava varieties (TMS 98/0505, TMS 01/1368, TMS 98/0510, TMS 30572, TME EB419, TMS 95/0289, TMS 98/0581) that were inoculated with either 30,000 or zero *M. incognita* eggs and grown for six months in sterilized soil dispensed into 30-liter plastic pots in a Randomized Complete Block Design with three replicates. In the field, eleven genera of plant-parasitic nematodes found were controlled with carbofuran (rate of 3 kg a.i./ha) in treated plots for comparison of cassava growth and yield in naturally-infected plots devoid of carbofuran. The 7x2 factorial experiment was replicated four times. At harvest, data were collected on plant height, stem girth, foliage weight, number and weight of root tubers, number of galls on feeder roots and tubers, number and identity of nematodes per given weight of feeder roots and soil. Infected plants were scored for galling on a 1-5 rating scale. Resistance to root-knot nematode in the pot experiment was based on Gall Index (GI), Nematode Reproduction Factor (R) and tuber yield. The result indicated that, galls were observed on feeder roots and also on the tubers of infected plants. Gall index varied from 3 to 5. TME EB419 variety was tolerant to *M. incognita* with average tuber yield of 425 g and 352 g/plant for inoculated and nematode-free cassava plants respectively; GI of 3.0 on a scale of 5.0 and 2.6 R. Six varieties (TMS 98/0505, TMS 01/1368, TMS 98/0510, TMS 30572, TMS 95/0289, TMS 98/0581) were susceptible to *M. incognita* with GI ranging between 4 and 5, R between 1.2 and 6.0, and significant average yield loss between 73-163 g/plant. The nematode infection significantly ($P<0.05$) reduced plant height and fresh tuber weight in the cassava varieties except for TME EB419. In the field, eleven genera of plant-parasitic nematodes (*Aphelenchoides*, *Tylenchus*, *Longidorus*, *Pratylenchus*, *Hoplolaimus*, *Rotylenchus*, *Helicotylenchus*, *Trichodorus*, *Xiphinema*, *Meloidogyne*, *Scutellonema*) were identified, with *Meloidogyne* being the most virulent. Carbofuran-treated plots had significantly ($P<0.01$) lower final densities of *Meloidogyne*, and no galls were observed on storage roots. The study concluded that parasitic nematode control significantly improved the growth and yield of the susceptible cassava varieties and reduced storage tuber rot. This suggests that cassava producers will benefit from planting the tolerant variety TME EB419 and the control of field nematodes where the other six have to be planted.

THE INFLUENCE OF ROOT DIFFUSATES AND AGE ON SPORE ATTACHMENT OF *PASTEURIA PENETRANS* TO THE CUTICLE OF ROOT-KNOT NEMATODES (*MELOIDOGYNE* SPP.). **Alake, G.¹, R.N. Perry^{2, 3}, K. Davies², and W.M.L. Wesemael^{3,4,5}.** ¹Dept. of Entomology and Nematology, Univ. of Florida, 1881 Natural Area Drive Gainesville FL 32611, USA, ²Dept. of Biological and Environmental Sciences, Univ. of Hertfordshire, Hatfield, Hertfordshire, AL10 9AB, UK, ³Ghent University, Faculty of Sciences, Department of Biology, Ledeganckstraat 35, B-9000 Ghent, Belgium, ⁴Institute for Agricultural and Fisheries Research (ILVO), Burg. Van Gansberghelaan 96, B-9820 Merelbeke, Belgium, ⁵Ghent University, Faculty of Bioscience Engineering, Laboratory for Agrozoology, Coupure links 653, B-9000 Ghent, Belgium.

The attachment of spores of *Pasteuria penetrans* to the cuticle of the second-stage juveniles (J2) of root-knot nematodes represents a fundamental process in the development and reproduction of the bacterium. We hypothesized that root diffusates will promote more spore attachment when compared with distilled water and that with aging of J2 attachment will progressively decrease. This study investigated the effects of root diffusates from tomato and maize. Spores of *P. penetrans* (strain PpBl, isolated from *Meloidogyne incognita*) were obtained from Rothamsted Research, Harpenden, UK. Attachment was examined on J2 of *M. fallax*, *M. chitwoodi* and *M. minor*. Tubes containing the nematode-*Pasteuria* mixture were centrifuged at 15,000 g for 5 min to initiate spore attachment. J2 were pre-treated with tomato and maize root diffusates for 2 h before attachment test. Spore attachment was achieved for the three species with more spores being attached to the cuticle of *M. minor* compared to *M. chitwoodi* and *M. fallax*. J2 age influenced the number of spores attached to the cuticle of the three *Meloidogyne* species. More spores were found attached to freshly hatched J2 of the three species compared to 7 and 14-day-old J2 but for *M. minor* this was not significant. When freshly hatched J2 were exposed to tomato root diffusates, *M. chitwoodi* and *M. fallax* showed fewer spores attached, whereas for maize diffusates only *M. fallax* showed fewer spores attached compared to distilled water. On 7 and 14 day-old-J2 of these species exposure to root diffusates did not influence

spore attachment. The highest numbers of spores were found attached to freshly hatched J2 of *M. minor* after exposure to tomato root diffusates. Diffusates from maize had no effect for *M. minor*. The high number of spores recorded for *M. minor* probably indicated the species-specificity in the attachment profiles. It is possible that substances present in the root diffusates favourably mediated the positive interaction between the cuticle surface of the nematodes and the endospore-binding surfaces. The disparity in the surface cuticle among the three *Meloidogyne* species used in this study may serve to explain their differing responses to the *P. penetrans* (strain PpBI).

EFFECT OF BIOLOGICAL SEED TREATMENTS ON *ROTYLENCHULUS RENIFORMIS* MANAGEMENT. Aljaafri, W.A.¹, G.W. Lawrence¹, V.P. Klink², and D.H. Long³. ¹Department of Biochemistry, Molecular Biology, Entomology & Plant Pathology. ²Department of Biological Sciences, Mississippi State University, Mississippi State, MS, 39762. ³Albaugh, LLC, 4060 Dawkins Farm Drive, Olive Branch, MS, 38654.

One recent strategy for nematode management is the application of biological control products. Biological control is being accepted as an alternative to chemical methods due to less negative effects placed on the environment. Experiments were conducted in the greenhouse at the R.R. Foil Plant Science Research Center at Mississippi State University to determine the efficacy of selected potential biological control products to manage *Rotylenchulus reniformis* on cotton and soybean. Experiments include three separate tests in which the biological compounds were applied to cotton and soybean as seed treatments. Seed applied products were received and treated by Albaugh, LLC. The study included the effect of these products on nematode life stage development. Seeds were planted in 500 cm³ of a steam sterilized sand: soil mix (1:1/ V: V) in 10 cm dia clay pots. Seeds were placed into one 2.54 cm depression in each pot with the addition of 2500 *R. reniformis* vermiform life stages. Tests included seeds treated with the standards Abamectin, ILeVo and a non-treated control. Treatments were arranged as a randomized complete block design with five replications. Tests ran for 50 days. On cotton, seeds treated with Abamectin and ALB-EXP5-1+ALB-M305-1 (7+ 3 fl. oz/cwt) significantly reduced the numbers of *R. reniformis* eggs recovered from the cotton roots. Eggs recovered from Abamectin and ALB-EXP5-1+ALB-M305-1 (7+ 3 fl. oz/cwt) seed treatments were 3347.4, 3862.4 respectively with 8755 eggs from the non-treated control. Seeds treated with Abamectin and ALB-M305-3 (10 fl. oz/cwt) had fewer juveniles and vermiform adult lives stage recovered from the soil compared with the non-treated seeds. *R. reniformis* was reduced 8.2 and 10.5% when treated with Abamectin, and ALB-M305-3, respectively compared with the control. On soybean, seeds treated with Abamectin and MBI305-3+ ALB-SAR 2 (7+ 0.25 fl. oz/cwt) significantly reduced the number of eggs recovered compared with the non-treated control. Eggs were reduced 29.411 and 35.294% in the Abamectin and MBI305-3+ ALB-SAR 2 treatments, respectively, compared with the non-treated control. Abamectin, ALB SAR (0.01 fl. oz/cwt), MBI305-3+ ALB-SAR 2(7+ 0.25 fl.oz/cwt) and ALB-M305-1(7 fl.oz/cwt) significantly reduced the number of vermiform life stages that were found in the soil compared with the non-treatment. No negative effects were recorded from any biological treatment on cotton or soybean in these tests.

MOLECULAR DETECTION AND IDENTIFICATION OF *HETERODERA GLYCINES* IN SOIL DNA EXTRACTS IN NORTH DAKOTA. Baidoo, R. and G.P. Yan. North Dakota State University, Department of Plant Pathology, Fargo, ND 58108.

The soybean cyst nematode (SCN) *Heterodera glycines* is a major pathogen of soybean worldwide. SCN belongs to the *H. schachtii sensu stricto* group which includes species only differing in minor morphological and morphometric characters. Distinction between SCN and other *schachtii* members such as *H. schachtii* and *H. trifolii* based on morphology using traditional microscopic methods is not only difficult and time consuming but also requires a high level of taxonomic expertise. Molecular techniques provide alternative means for distinguishing these nematodes species. The study was therefore conducted to utilize molecular procedures to differentiate SCN from *H. schachtii* and other closely related cyst nematodes that may occur in North Dakota (ND), and to develop a molecular assay to detect SCN sensitively and directly in field soils with low population density. The MO BIO PowerSoil[®] DNA Isolation Kit was used to extract total genomic DNA in triplicates from infested soils. Detection sensitivity was determined by the ability to detect a minimum number of eggs or juveniles/g of soil. Different numbers of SCN eggs or juveniles (n = 0, 1, 2... 8) were inoculated into 10 g of sterilized SCN-free field soil. DNA was extracted from 0.25 g of the infested soil and a specific amplicon of 477 bp was amplified using a published SCN-specific primer set SCNF1/SCNR1. DNA extracts of 37 other plant-parasitic nematodes were used to confirm primer specificity. The result showed that the PCR assay could detect up to 1 SCN egg or juvenile/10g of soil representing 20 eggs or juveniles/200g of soil. The PCR assay was validated by comparing its detections in fields positive for SCN as determined by the traditional method. A total of 27 soil samples from soybean, corn and wheat fields in ND harboring a range of SCN population densities (0 to over 17,000 eggs/200 g of soil) were collected for the experiment. SCN identification was confirmed by sequencing two genomic regions of 15 populations. For each soil sample, 400 g of soil was collected and divided into two for molecular detection and traditional eggs extraction and quantification. The PCR assay detected SCN in all three independent DNA extractions from the top 10 most SCN-infested fields (≥ 300 eggs/200 g of soil). Although, SCN detectability was lower and inconsistent at low

densities (<200 eggs/200 g of soil), grinding the field soil before DNA extraction and PCR inhibitors removal by $\text{NH}_4\text{Al}(\text{SO}_4)_2$ treatment followed by nested PCR improved detection efficiency by 100%, thus, enabled SCN detection in all the infested fields up to the lowest density 12 SCN eggs/200 g of soil. The PCR assay could differentiate SCN from other *schachtii* members and provides a sensitive and efficient detection of SCN at low population density useful for field screening or laboratory detections of SCN, obviating the time-consuming steps of nematode extraction, microscopic identification and counting.

ENTOMOPATHOGENIC NEMATODE HOST-SEEKING BEHAVIOR AND TEMPORAL ATTRACTION TO NAÏVE AND INFECTED HOSTS. **Baiocchi, T. and A.R. Dillman.** Department of Nematology, University of California, Riverside, California 92521.

Entomopathogenic nematodes (EPNs) are insect parasites that are used as biological control agents, providing ecologically friendly alternative to chemical pesticides. The infective juveniles (IJs) of EPN species are free-living and employ host-seeking behaviors such as chemotaxis to locate suitable hosts for infection. Chemosensory information plays a central role in the decision of whether or not to infect a potential host. Furthermore, chemo-sensation may reveal if a potential host is already infected with EPNs (either conspecific or hetero specific species). The health status of a potential host can greatly impact the reproduction potential and overall fitness of nematodes infecting a particular host. Infecting a naïve host incurs the risk of failure to overcome the immune response, or the possibility that mates may not be encountered. An infected host poses several benefits – host immune response may be overcome and potential mates may be present – but at later stages of an infection resources may be low and thus could greatly reduce reproduction potential and success of a newly invading IJ. We used an assay designed to specifically test volatile odorants and tested the attraction of several *Steinernema* species (*S. carpocapsae*, *S. feltiae*, *S. glaseri*, and *S. riobrave*) to naïve and infected hosts over time. We found that the attractiveness of infected hosts to IJs changes over time in a species-specific manner. This may be based on the foraging strategy of the nematodes. Furthermore, we found that EPNs can differentiate between naïve and infected hosts, and that hosts infected for many days become repulsive to foraging IJs. We are currently using solid phase micro-extraction (SPME), gas chromatography, and mass spectrometry (GCMS) to identify the odorants informing IJ attraction to infected hosts and how these odor profiles change over time.

DISTRIBUTION AND GENETIC DIVERSITY OF *ANGUINA* SPP. IN THE PACIFIC NORTHWEST, UNITED STATES. **Barrantes-Infante, B.L.¹, B.K. Schroeder², and T.D. Murray¹.** ¹Department of Plant Pathology, Washington State University, Pullman, WA 99164, USA, ²Department of Plant, Soil and Entomological Sciences, University of Idaho, Moscow, ID 83844, USA.

The genus *Anguina* is a specialized group of plant parasitic nematodes associated with lesions on stems and seeds in grasses and small grain cereals. *Anguina* species can act as vectors of *Rathayibacter* spp. bacteria, carry them into plants, resulting in gummosis diseases of grasses and wheat worldwide. Both the nematodes and the bacteria are threats to agriculture in many countries. Little is known about the incidence and distribution of *Anguina* species in native grasses or those under cultivation in the United States and their potential associations with *Rathayibacter* spp. This study aimed to better understand the prevalence and distribution of *Anguina* species in the Pacific Northwest (PNW) region of the U.S. and consequently of associated *Rathayibacter* spp. We used nuclear and mitochondrial markers to estimate the genetic diversity of *Anguina* spp. in the PNW, especially those associated with the grass host *Sporobolus cryptandrus* in west central Idaho. A total of 189 samples of grasses were collected throughout the PNW (2013-2014) and in Idaho (2015). Samples of *Agropyron smithii* from New Mexico and *Agrostis* sp. from Washington State were also used. Forty-six of the 189 samples were positive for *Anguina* spp. and were collected from 25 sites near White Bird, Idaho. All of these grasses were identified as *S. cryptandrus*. Sequencing of the nuclear ribosomal ITS region, from the nematodes collected from *S. cryptandrus*, revealed two alleles (Ai1 and Ai2) separated by a single polymorphic site. Based on Bayesian phylogenetic analyses that included GenBank sequences from described and undescribed *Anguina* spp., nematodes carrying these alleles fell into a single well-supported clade with sequences from *Anguina wevelli* (accession numbers AM888393 and AF396317) and two undescribed *Anguina* species (accession numbers AF396316 and KM114441). Furthermore, a subclade is formed with the nematodes carrying the alleles Ai1 and Ai2 and *Anguina* sp. (KM114441). Supportive work, such as morphological and morphometric data are needed to fully describe this species. Nematodes extracted from *A. smithii* and *Agrostis* sp. were each found in a monophyletic group with *Anguina agropyronifloris* (accession number AF363093) and *Anguina agrostis* (accession numbers KM114436, AF396338, AF396344, AF396339, KM114437 and AM888391), respectively. Partial sequences of the mitochondrial *coxI* gene revealed considerably more genetic variation than ITS among nematodes from Clade III and resulted in seven haplotypes among nematodes associated with *S. cryptandrus*. A minimum spanning network revealed seven haplotypes with Haplotypes 1 and 2 representing 37 and 35%, respectively, of sampled nematodes and separated by one mutation. A third haplotype represented 15% of sampled nematodes and was separated from Haplotype 2 by 32 mutations, suggesting that this haplotype may represent a cryptic species.

MELOIDOGYNE INCOGNITA EMIGRATION FROM COTTON ROOTS MAY BE INDUCED BY THE RESISTANCE QTL *QMI-C11*. Batista da Silva, M.¹, P. Kumar¹, B. Nichols⁴, P.W. Chee¹, and R.F. Davis². ¹University of Georgia, Tifton, Georgia; ² USDA-ARS Crop Protection and Management Research Unit, Tifton, Georgia; ⁴Cotton Incorporated, Cary, North Carolina.

Upland cotton (*Gossypium hirsutum*) is one of the most widely grown crops in the southern US, and *Meloidogyne incognita* is the most significant pathogen of cotton in the US. Two QTLs, *qMi-C11* and *qMi-C14*, conferring resistance to *M. incognita* have been identified in cotton. Previous research documented resistance expressed at two stages of nematode development, and later research documented an epistatic interaction between the two QTLs, both of which suggest the QTLs have different modes of action. Our objective was to document the effects of *qMi-C11* and *qMi-C14* on *M. incognita* penetration, development, and reproduction in cotton. We developed near-isogenic lines (NIL) carrying only a single QTL and observed *M. incognita* development in NILs containing both QTLs (M-120), only one QTL (NIL-C11 with *qMi-C11* or NIL-C14 with *qMi-C14*), or neither QTL (Coker 201). Compared to the susceptible Coker 201, NIL-C11 stopped many nematodes from developing beyond the SJ2 stage whereas NIL-C14 limited the development of J4 into females, and both consequently reduced egg production. Approximately 50% of the nematodes in NIL-C11 and M-120 plants remained in stage J2 or SJ2 25 days after inoculation (DAI) compared to 12% of Coker 201. The number of nematodes in the roots did not differ among genotypes 4 and 8 DAI, but were lower in NIL-C11 and M-120 after 8 DAI, which may indicate *M. incognita* emigration. For additional studies, we hypothesized that on resistant lines 1) J2s are failing to establish a feeding site and leaving the roots, and 2) *M. incognita* is producing fewer eggs/egg mass. To test our hypotheses, two-week-old seedlings were inoculated; two days later, roots were rinsed and seedlings were transplanted into small cones. On each of five sampling dates (4, 6, 8, 10, and 12 DAI), nematodes that had left the roots were extracted from vermiculite and roots were stained to count nematodes inside the roots. NIL-C11 had more nematodes leaving the roots on 6 and 12 DAI and M-120 had more on 4, 8, and 10 DAI than Coker 201. By the end of the experiment, more than 50% of the nematodes were in vermiculite rather than in the roots for NIL-C11, while about 10% were in vermiculite for Coker 201. To measure eggs/egg mass, two-week-old seedlings were inoculated, and 30 DAI, eggs from 10 egg masses from each genotype in each replicate were counted. Eggs/egg mass did not differ among genotypes. We conclude that low levels of nematode emigration occur on susceptible plants, but *qMi-C11* causes many J2s to leave the roots or fail to develop beyond the SJ2 stage. In contrast, *qMi-C14* does not stimulate significantly more nematode emigration but causes many nematodes to fail to develop beyond the J4 stage.

EVALUATION OF NOVEL NEMATOCIDES FOR CONTROL OF ROOT-KNOT NEMATODES IN PROCESSING TOMATO. Becker, O.¹, A. Ploeg¹, and J. Nunez². ¹Dept. Nematology, University of California, Riverside, CA 92521, ²Cooperative Extension Kern County, Bakersfield, CA 93307.

California produces more than 90% of US processing tomatoes on about 118,000 hectares. Since 1980 the average yield has steadily increased from 10 to 20 tons per hectare. Root-knot nematodes (rkn), in particular *Meloidogyne incognita* and *M. javanica*, are widely distributed in tomato production fields but they have been managed successfully for many years through Mi-resistant cultivars. The increasing occurrence of Mi-gene resistance-breaking rkn populations is a growing problem not only because of the yield damage potential but also because of the danger of wider dissemination of these strains. Soil fumigant use is generally limited by regulatory restrictions related to their negative impact on air quality and environmental toxicity. For several years we have field-tested novel non-fumigant nematicides in *M. incognita*-infested sandy loam soils in Southern California. Fluensulfone (Nimitz®) was either pre-plant soil incorporated or chemigated via drip irrigation seven days before planting. Fluopyram (Velum®) was applied as a split application at planting and two weeks later. Both products reduced root galling of the rkn-susceptible tomato cultivar Halley 3155 at mid-season and at harvest. They also mitigated secondary microbial decay. This resulted in significant yield increases compared to the non-treated control. Both products are considered environmentally safer than organophosphate and carbamate nematicides.

A SCANNER ASSAY DEVELOPED TO QUANTIFY NEMATODE POPULATION MOVEMENT AND ITS APPLICATIONS FOR NEMATOCIDE SCREENING. Beeman, Augustine Q.¹, Z.L. Njus², S. Pandey², and G.L. Tylka¹. ¹Department of Plant Pathology and Microbiology and ²Department of Electrical and Computer Engineering, Iowa State University, Ames, IA 50011.

Traditionally, the sensitivity of nematodes to nematicides has been measured through microscopic observations of nematode movement after exposure to compounds. While using this assay can be quick and inexpensive, there are drawbacks to this approach. A compound may inhibit nematode motility temporarily, only to wear off with little or no lasting effect on nematode fitness. Additionally, a compound may not have a visually obvious effect on nematode motility but may affect the ability of nematodes to travel meaningful distances. The objective of this research was to develop an assay that scans individual wells of tissue-culture plates containing small populations of nematodes at specified intervals automatically and uses the images to quantify nematode population movement. A scanner enclosed in a temperature-controlled acrylic box was used to develop and test the assay. A computer program was written to scan the wells containing nematodes in sterile distilled water and capture high-resolution (2400 dpi) images. After scans are completed, the program takes the series of images from

a single well and divides each image into several frames, and a user identifies the nematodes in the image at each time point. Selected points on each nematode are converted by the software to x and y coordinates, and the program calculates the distance (in μm) that each nematode in a well has traveled since the last image that was collected. Nematodes whose position changed by 200 μm or more between images are classified as having moved, and the percentage of nematodes moving in the whole well is quantified. Initial experiments using heat-killed and unheated second-stage juveniles of the soybean cyst nematode, *Heterodera glycines*, revealed drift of dead nematodes in the water being recorded as movement. Using 11.5% Pluronic gel instead of water minimized this drift. The nematicide abamectin was used to further validate the assay. *Heterodera glycines* juveniles were incubated in 0, 0.1, 1, 10 and 100 mg/L abamectin for two hours and subsequently washed three times with sterile distilled water. An average of 30 juveniles were placed in wells of a 24-well plate containing 11.5% Pluronic gel and scanned every hour for 24 hours, with six replications per treatment. The experiment was repeated once with similar results. Movement of *H. glycines* juveniles in 0 and 0.1 mg/L abamectin was similar, with approximately 65% of the juveniles moving at 2 hours and movement gradually reduced to around 35% after 24 hours. Abamectin concentrations of 1, 10 and 100 mg/L reduced movement of *H. glycines* juveniles to 9.8%, 5% and 2.7%, respectively, after abamectin exposure ($P < 0.0001$). The scanner assay was useful in measuring the response of *H. glycines* to abamectin, and current work is underway to adapt the assay to study *H. glycines*-soybean root interactions.

SOYBEAN APHID FEEDING AFFECTS SOYBEAN CYST NEMATODE EGG HATCHING *IN VITRO*. Beeman, A.Q. and G.L. Tyka. Department of Plant Pathology and Microbiology, Iowa State University, Ames, IA 50011.

The soybean cyst nematode, *Heterodera glycines*, and the soybean aphid, *Aphis glycines*, are economically important pests of soybean. Recent research has shown that *A. glycines* feeding affects *H. glycines* reproduction, although the mechanism behind the interaction is not currently understood. The purpose of this research was to determine if *A. glycines* feeding on *A. glycines*-resistant and susceptible soybean cultivars affects hatching of *H. glycines* eggs *in vitro*. Near isogenic soybean cultivars that were aphid susceptible and aphid resistant with the Rag1 resistance gene were planted in a pasteurized 2:1 sand: soil mixture and grown in a growth chamber (25 °C, 16:8 hour light:day). Seven days after planting (DAP), the plants were transferred to 1 liter plastic boxes containing quarter strength Murashige and Skoog medium with Gamborg's vitamins and grown hydroponically in a randomized complete block design (RCBD). At 14 DAP the plants were infested with 0, 10 or 20 apterous *A. glycines* (biotype 1, controlled by Rag1), covered with netting to prevent aphid movement across plants, and grown until root exudate collection at 21 DAP. Exudates were collected by gently rinsing the hydroponic medium from the roots with water and incubating the roots in distilled water for 24 hours. Root exudates were filter sterilized and used for *H. glycines* hatching experiments. Hatching experiments were conducted as a two-factor factorial with three levels of aphid density and two levels of soybean cultivar. Approximately 300 to 400 *H. glycines* eggs were placed on microsieves and incubated in one of the six *A. glycines* density by soybean cultivar treatment combinations, or in a positive (5 mM ZnSO₄) or negative (sterile distilled water) control. Eggs were incubated at 25 °C in darkness in a RCBD design with five replications, and the experiment was repeated once with similar results. The number of hatched *H. glycines* second-staged juveniles that had moved through the microsieves and into the solution was counted at 3, 7 and 14 days, and the number of remaining unhatched eggs remaining on the sieves was counted at 14 days to determine calculate cumulative percent hatch. Root exudates from all treatments stimulated hatching ($P < 0.0001$) compared with water (49% versus 36%), while ZnSO₄ stimulated hatching ($P < 0.0001$) compared to root exudates (62% versus 49%). There was no effect of cultivar on hatching ($P > 0.05$), therefore hatching of *H. glycines* in root exudates collected from aphid-resistant and susceptible soybeans were combined for analysis. Exudates collected from soybeans infested with 10 and 20 aphids reduced ($P = 0.0121$) *H. glycines* hatching relative to the uninfested control (46% and 47%, respectively, relative to 53% in the uninfested control). These results indicate that feeding by *A. glycines* aboveground may alter soybean root exudates, ultimately resulting in modest reductions of *H. glycines* egg hatching.

CONSERVATION AGRICULTURE – DO WE GET BETTER YIELDS AND WHAT IS BEHIND IT? Bekker, S¹, Daneel, M², Fourie¹, H and Nel, A³. ¹Unit for Environmental Sciences and Management, North-West University, Private Bag X6001, Potchefstroom, 2520, South Africa, ²Agricultural Research Council – Institute for Tropical and Subtropical Crops (ARC – ITSC), Private Bag X11208, Nelspruit, 1200, South Africa, ³Agricultural Research Council - Grain Crops Institute (ARC – GCI), Private Bag X1251, Potchefstroom, 2520, South Africa.

Conservation Agriculture (CA) is based on minimum soil disturbance, crop rotation and soil cover and is expected to result in increased yields, soil fertility and health. The objective of this study was to determine the effect of CA on maize yield in different maize-cropping sequences. This included monoculture maize produced under conventional practices (CT) compared with monoculture maize and maize rotated with cowpea/sunflower only or cowpea/sunflower and pearl millet sequence under CA practices. These trials were conducted over a four-year period in two different sites, Buffelsvallei (sandy loam soil) and Erfdeel (sandy soil) as part of a CA programme of the ARC – GCI conducted in the North West and Free State provinces of South Africa. Annual maize yield was determined while nematodes and soil nutrients were also sampled annually. At the end of the trial, average maize yield per treatment was determined to compare the effect of the different crop rotation

sequences on yield. Multi-table analysis was furthermore performed to determine the effect of plant-parasitic nematodes, soil chemistry and crop rotation sequences on yield over the 4-year period, allowing four years to be compared in one graph known as a compromise. At Erfdeel, plant-parasitic nematodes seemed to have a greater impact on yield than soil chemistry, with plots with higher *Pratylenchus*, *Nanidorus* and *Meloidogyne* numbers showing the lower yields. This coincided with monoculture maize and maize/cowpea/pearl millet/maize rotations. On the other hand, treatments with two cowpea rotations seemed to have higher yields which correlated with lower nematode numbers in the cowpea sequences. At Buffelsvallei, plant-parasitic nematodes also seemed to have a greater impact on yield than soil chemistry. Plots in which *Meloidogyne* dominated, had lower yields and were mostly recorded for monoculture crop sequences. Interestingly high *Scutellonema* and *Rotylenchulus* numbers were correlated with higher yields coinciding with crop rotation treatments. It is important to gather data over a longer period to confirm these results and determine the effect of CA on crop productivity.

NEMATODE COMMUNITIES IN CONSERVATION VERSUS CONVENTIONAL AGRICULTURAL PRACTICES: A SOUTH AFRICAN SCENARIO. Bekker, S.¹, H. Fourie¹, M. Daneel² and A. Nel³. ¹Unit for Environmental Sciences and Management, North-West University, Private Bag X6001, Potchefstroom, 2520, South Africa, ²Agricultural Research Council – Institute for Tropical and Subtropical Crops (ARC – ITSC), Private Bag X11208, Nelspruit, 1200, South Africa, ³Agricultural Research Council - Grain Crops Institute (ARC – GCI), Private Bag X1251, Potchefstroom, 2520, South Africa.

Conservation agriculture (CA) primarily aims to optimise crop production by applying practices and principles that promote soil quality. This is mainly done by increasing population levels and/or the diversity of beneficial organisms of which non-parasitic nematodes (NPN) form part of. The objective of the study was to determine and monitor nematode communities over four growing seasons in both CA and conventional (CT) systems. Two rain-fed field trial sites, Buffelsvallei (sandy loam soils) and Erfdeel (sandy soils) (part of a CA programme of the ARC – GCI conducted in the West Province), where different maize-cropping sequences were included were used for the purpose of this study. The cropping systems represented maize monoculture (CA and CT) and different combinations of maize with cowpea, pearl millet and sunflower. Nematode samples were obtained during each growing season from all plots before planting (soil only) as well as 60 (soil and roots) and 100 (soil and roots) days after planting. Nematode population levels increased substantially from zero to 100 days after planting. *Rotylenchulus parvus* dominated in roots of crops at Buffelsvallei and *Meloidogyne incognita* at Erfdeel. Cropping sequences in which cowpea were included had significantly lower *R. parvus* and *M. incognita* population levels, while those in which sunflower were included showed the same tendency for *R. parvus*. In terms of NPN, *Aphelenchus* dominated at Buffelsvallei and *Acrobeloides* at Erfdeel. This indicated that fungal and bacterial decomposition channels existed at the two respective sites 100 days after planting. Significant interactions for season x nematode abundance (PPN and NPN) were evident at both localities. Generation of nematode data over a longer period should be done to obtain more knowledge about both biotic and abiotic factors that impact on the restoration of soil quality and sustainable crop productivity.

NEMATODE COLONIZATION OF NEWLY EXPOSED LAND SURFACES. Bernard, E.C. Entomology & Plant Pathology, University of Tennessee, 2505 E. J. Chapman Drive, 370 Plant Biotechnology, Knoxville, TN 37996-4560.

Whether nematodes can be defined as true extremophiles is open to debate if the benchmarks are bacteria living in pressures and temperatures unsuited to eukaryotes. As stated in NOAA's definition of extremophiles, "...these organisms are 'extreme' only from a human perspective." Nematodes exhibit adaptations such as dauer juveniles, dormancy, other non-feeding stages and anhydrobiosis to survive conditions unfavorable to active life. How quickly and by what fashion do nematodes colonize newly exposed land such as new islands, deglaciated surfaces and reclaimed mines? What little evidence exists suggests that this process is remarkably rapid. Nematodes follow closely upon the heels of glacier recession, with Criconematina already present within 15 years of land exposure. Similarly, unconsolidated lava fields with only a few cracks for exploitation by plants also tend to have *Paratylenchus* spp. feeding on those plant roots. Paratylenchidae have non-feeding fourth-stage juveniles, but Criconematina as a whole have no special dispersal abilities as far as is known. This problem relates to the recolonization of land following recession of the Laurentide ice sheet, as all major groups of soil nematodes were able to move northward and colonize northern North America. If current plant host and vertebrate data are accepted as valid for nematodes as well, then most plant parasites would have been pushed deeply to the south. Recolonization with glacier recession would have required crossing enormous river systems, formed by ice melt, mostly flowing the wrong way (south, east or west). The usual scenario for survival near glaciers is that it would have been extremely difficult, but this scenario usually is related to vertebrate survival. Nematodes may well have kept up by dispersal close to the receding glacial face, with plant parasites appearing a few years after hardy vascular plants. The main problem for nematodes may have been adaptations for periodic freezing, but the abundance of plant parasitic taxa above permafrost in Alaska demonstrates that this capability is widespread across the phylum. Evidence from reclaimed mine spoils shows that reconstitution of nematode communities to their original state is a rapid process that takes less than 20 years. Understanding nematode distributions to hostile environments requires a deeper knowledge of dispersal mechanisms than we now have.

FIRST REPORT OF A DORYLAIMID NEMATODE FROM THE INTESTINE OF A MILLIPEDE (DIPLOPODA). **Bernard, E.C. and G. Phillips.** Entomology & Plant Pathology, University of Tennessee, 2505 E. J. Chapman Drive, 370 Plant Biotechnology, Knoxville, TN 37996-4560.

A specimen of the millipede *Narceus americanus*, collected in Hocking County, Ohio, was dissected to determine the presence of gut nematodes. In addition to rhigonematid and thelastomatid nematodes, the intestine contained 10 specimens of Dorylaimida, including males, females and late-stage juveniles. The specimens key straightforwardly to the family Qud-sianematidae and *Labronemella* in standard manuals, but in the most recent realignment of Dorylaimida would be placed in Dorylaimidae, subfamily Labronematinae, as adults of both sexes have short, rounded tails. The Ohio specimens resemble *Labronemella* in having a sunken oral field, rather slender stylet, doubled guiding ring and similar male reproductive structures. However, the Ohio specimens differ significantly from other labronematines in having strong thickenings of the vestibule walls reminiscent of Actinolaimidae, but without the teeth present in that family. The males of this dorylaimid have strongly bent spicules, about 19 precloacal midventral supplements, nine pairs of widely spaced subventral supplements, and three pairs of sublateral supplements. Molecular analysis on one specimen suggests some affinity with *Nevadanema* (Dorylaimidae, Labronematinae), *Prodorylaimus* (Dorylaimidae, Prodorylaiminae) and *Paractinolaimus*. The latter genus is a member of Actinolaimidae; therefore, the Ohio specimens may represent a taxon with both dorylaimid and actinolaimid characteristics, strengthening the suggestion that the two families are sister taxa. The presence of these nematodes in a millipede is puzzling. These nematodes were well-embedded in the intestinal contents and were not moving, but they were intact and in good anatomical condition. They were much longer than the particles of plant material in which they were embedded. Therefore, if they had been eaten by the millipede they should have been bitten into pieces. If they are indeed capable of living in the intestine, they almost certainly prey on the abundant rhigonematid and thelastomatid nematodes found in the intestine of *N. americanus*.

ENDEMIC *OSCHEIUS* NEMATODES OF HAWAII. **Bisel, J.¹, R. Myers², and B. Sipes¹.** ¹Department of Plant and Environmental Protection Sciences, University of Hawaii, Honolulu, 96822 and ²Daniel K. Inouye USDA Pacific Basin Agricultural Research Center, Hilo, 96720.

Entomopathogenic nematodes (EPNs) parasitize insects utilizing mutualistic bacteria to kill the host, allowing the nematode to feed and reproduce within the insect cadaver. Consequently EPNs are highly sought after for their biological control potential. A survey for EPNs was conducted on O'ahu and Hawai'i Island using a modified baiting method. One hundred seven soil samples were collected and baited with five *Tenebrio molitor* (mealworm) larvae. Soil samples were observed daily for 5 days and morbid *T. molitor* larvae were placed on white traps. Forty-seven of the 107 locations contained at least one infected mealworm containing nematodes. Mealworm mortality was attributed to EPNs, fungal contamination, parasitoids or an unknown factor in 16%, 10%, 1% and 73% of samples respectively. Eighty-two EPN isolates were passed through two subsequent inoculations in order to confirm their entomopathogenic nature. A total of 41 EPN isolates were recovered through three rounds of reinoculation. PCR analysis and sequencing was conducted on third generation EPN, targeting the ITS region. *Oscheius* was recovered from 96% of locations sampled on Hawai'i Island and O'ahu respectively. Sequencing analysis suggested three groups of *Oscheius*. The *Oscheius* isolates and an unknown nematode isolate occurred in 76%, 12%, 8% and 4% of positive locations respectively. This survey suggests that *Oscheius* is a common EPN in Hawai'i.

COMPARISON OF EXTRACTION METHODS TO ACCURATELY ESTIMATE PLANT-PARASITIC NEMATODES FROM A VARIETY OF SOILS. **Blauel, T., T. Wallace, D. VanDyk, M. Celetti, M.R. McDonald. and K. S. Jordan.** Plant Agriculture Dept., University of Guelph, Guelph, ON, N1G 2W1.

Various management decisions regarding plant-parasitic nematodes are often made based on screening of soils for the presence of nematodes either prior to planting or as a result of unexplained symptoms on the plants. Published threshold levels are also used to determine whether or not to apply nematicides or even to fumigate soils to prevent nematode damage during a growing season. However, inconsistencies between laboratories has made it difficult for growers to have confidence in soil test results. Additionally, the traditional method of soil nematode extraction, the Baermann pan (BP) method, only extracts live, motile nematodes. It is possible that slow-moving genera are being excluded from counts or are being underestimated due to the nature of the extraction method. In order to extract the most nematodes, the BP method requires days before counts can be made. The sugar centrifugation (SC) method for nematode extraction from soils extracts both living and non-living nematodes but can also be used to estimate populations of slow-moving or non-motile genera in the soil. Another advantage is that the extraction and counts can all be done the same day that samples are collected, allowing growers to obtain a faster response. Although most diagnostic laboratories in Canada use the BP method for soil nematode extraction, it is believed that for certain genera the SC method more accurately estimates population levels. A series of studies were undertaken to compare nematode extraction methods from soil to ensure that current recommendations are based on the most accurate predictions of nematode populations in the soil. Samples from turfgrass (representing turfgrass nematode genera and sandy soils), carrot (representing carrot genera and high organic matter (muck) soils) and tomato (representing tomato nematode genera and fine-textured soils) were collected and subjected to both the BP and SC soil nematode extraction

methods. From turfgrass soils, 3 golf greens sites were selected at 4 different golf courses for comparison. From the carrot soils samples from five different fields were collected and from the tomato fields, samples from ten different fields were collected for comparison. All samples were homogenized prior to removing 50cc aliquots of soil for extraction. In the turfgrass samples, the SC method extracted significantly more nematodes within the *Heterodera*, *Meloidogyne*, *Criconeoides*, *Helicotylenchus* and *Tylenchorhynchus* genera but there was no significant difference between the two methods for free-living nematodes. In the muck soil samples, the SC method extracted significantly more nematodes within the *Heterodera* genus but significantly fewer *Paratylenchus* nematodes. Additional analysis from carrot and tomato soils is currently underway.

DEVELOPING POTATOES WITH BROAD SPECTRUM RESISTANCE TO *GLOBODERA*. Blok, V. The James Hutton Institute, Invergowrie, Dundee, UK DD2 5JQ.

Host resistance has been shown to be a highly effective management tool for suppressing and limiting the spread of pathogens and pests. In the UK, popular cultivars such as Maris Piper which has *HI* resistance derived from *Solanum tuberosum* ssp. *andigena*, have now been grown for several decades. This has led to a reduced occurrence of *Globodera rostochiensis*; however, during this period, *G. pallida* has become more prevalent and wide-spread. The genetic complexity of *G. pallida* field populations in UK field populations, which are typically comprised of more than one molecularly distinct introduction, has raised concerns about the durability of resistance from a single source to this species. In addition, breeding of agronomically acceptable cultivars with resistance to *G. pallida* has been problematic as a single major gene resistance to the predominant UK pathotypes of this species has not been identified. Never-the-less, recently cultivars with high levels of resistance to *G. pallida* derived from *S. vernei* (*GpaV*) and *S. tuberosum* ssp. *andigena* (*GpaIV^s_{adg}*) have become available to the industry and their performance is being evaluated both in glasshouse trials using *G. pallida* populations with different virulence characteristics and in the field. The results are promising; however, the potential for selection for increased virulence of *G. pallida* remains a concern. Hence, breeding programs are now generating potato genotypes in which these resistances have been combined with the aim of achieving broad-spectrum and durable resistance.

NEMATODE COMMUNITY ANALYSIS IN DRY LAND CEREAL PRODUCTION SYSTEMS OF CENTRAL MONTANA. Briar, S.¹, A. Burkhardt², J. Sherman², P. Carr¹, and D. Wichman¹. ¹Central Agricultural Research Center, Montana State University, Moccasin, MT 59462, ²Department of Plant Sciences and Plant Pathology, Montana State University, Bozeman, MT 59717.

Previous surveys focusing on plant-parasitic nematodes on dryland farming systems documented that root-lesion nematode has become a serious pathogen under continuous cereal production systems in central Montana (Johnson, 2007). However, no information is recorded on the presence and abundance of non-parasitic species that form part of the total nematode complex in the soil-root interface of cereals. Nematode community and soil properties were analyzed from 12 different fields covering 6 locations under cereal production system in central Montana. Eighteen nematode genera were identified. Total nematode abundance ranged from 120 to 891 per 100g soil dry weight. All the fields were positive for plant-parasitic root lesion nematode. These data suggest that continuous cropping of susceptible wheat and barley cultivars can lead to economically damaging populations of root lesion nematode. A screening of lines from the Barley World Core for root lesion resistance is underway to genetically dissect novel germplasm for resistance and utilize newly identified genes to improve barley for nematode resistance. Preliminary results of soil nematode faunal analysis based on enrichment (EI) and structure indices (SI) have revealed moderately to highly enriched but moderately to low structured soil food webs in general. Nematode community analysis indicates very low SI values and a low population of late succession high c-p value nematodes (omnivorous and predatory spp.) in two fields with high levels of exchangeable aluminum (Al) and low soil pH, suggesting a lack of trophic links possibly due to negative impacts of high levels of Al to the soil food web. Farming practices aimed at amending soils and mitigating the impacts of Al on soil food web and nematode community structure linkages needs to be investigated further.

SPECIES OF *MELOIDOGYNE* AND OTHER PHYTOPARASITIC NEMATODES IDENTIFIED IN FLORIDA PEACH ORCHARDS. Brito, J.A.¹, S.A. Subbotin², D.W. Dickson³, R.N. Inserra¹, T. Smith¹, J.S. Vau³, S. Qiu³, L.W. Duncan⁴, and J.D. Stanley¹. ¹Division of Plant Industry, DPI- FDACS, Gainesville, FL 32614-7100; ²Plant Pest Diagnostic Center, California Department of Food and Agriculture, Sacramento, CA 95832-1448, ³Entomology and Nematology Department, University of Florida, Gainesville, FL 32611-0620, ⁴University of Florida, IFAS, Citrus Research and Education Center, Lake Alfred, FL 33850.

A total of 130 soil and root samples of peach (*Prunus persica*) were collected in 2016 from 10 orchards located in central and southern Florida. Root-knot nematode species were identified using isozymes and sequencing of two fragments of mtDNA, as well as the morphology of the perineal patterns when needed. Diagnostics of other plant-parasitic nematodes were made based on morphology of selected characters and sequencing of ribosomal RNA genes. Root-knot nematode species found were: *Meloidogyne arenaria* (H3 phenotype), *M. floridensis*, and *M. javanica*. The taxonomic status of

M. arenaria (H3 phenotype) is still under study. The peach root-knot nematode, *M. floridensis* was found in three orchards, each located in Alachua, Charlotte and St. Lucie Counties. In Charlotte Co., it was found in a mixture with *M. javanica*. Five orchards were infested with *M. javanica*, two in Pasco, and one each in Charlotte, Polk and Sumter Counties. Root-knot nematode, *M. arenaria* (H3 phenotype) was identified from three orchards in Polk Co., and in one of these orchards it was found mixed with *M. javanica*. The ring nematode *Mesocriconema xenoplax*, was found in seven orchards located in Alachua (1), Charlotte (1), St. Lucie (1), Pasco (1) and Polk (3) Counties. In Charlotte, St. Lucie, Pasco and Polk Counties, the lesion nematode, *Pratylenchus brachyurus* was found infecting peach roots. The morphological identifications of the lesion and ring nematodes were validated by the molecular analyses. Both species were previously reported by Malo in 1963 infecting selected peach rootstocks grown in some Florida Counties. Other plant-parasitic nematodes detected include: *Belonolaimus longicaudatus* (Polk Co.), *Hemicaloosia vagisclera* (Charlotte Co.), *Hemicyclophora weyi* (Charlotte Co.), *Mesocriconema ornatum* (Polk Co.), *Pratylenchus scribneri* (Polk Co.), *Paratrichodorus allius* (Charlotte Co.), *P. minor* (Polk Co.), *P. renifer* (St. Lucie Co.), *Tylenchorhynchus* sp. (Charlotte and Pasco Counties), *Xiphinema citricolum* (Pasco Co.) and *X. vulgare* (Polk Co.). The latter listed nematodes might be parasites of grasses and weeds growing around peach trees.

MICROBIAL-MICROBIAL INTERACTIONS IN THE GUT: HUMAN MICROBIOTA DISRUPTED BY GIARDIA ARE LETHAL TO *C. ELEGANS*. Buret, A.G.¹, D. Hansen¹, T. K. Gerbaba². ¹University of Calgary, Biological sciences, Inflammation Research Network, Host-Parasite Interactions. ²Queen's University, Medicine, Gastrointestinal Diseases Research Unit.

Microbiota dysbiosis has been implicated in a broad range of disorders, including Inflammatory Bowel Disease (IBD) and post-infectious Irritable Bowel Syndrome. The mechanisms remain unclear, and cause-to-effects studies are lacking. The protozoan parasite *Giardia duodenalis* is one of several enteropathogens (including *Campylobacter jejuni*) for which post-infectious complications have been reported. We recently reported that exposure to *C. jejuni* activates latent virulence genes in non-pathogenic *E. coli* (1). Our hypothesis was that exposure to an enteropathogen during the acute phase of infection, may induce virulence in gut microbiota, and that this pathogenic microbial-microbial interaction may be detected as lethal toxicity in *C. elegans*. To assess the effects of bacteria exposed to Giardia on *C. elegans*. Incubation with *E. coli* exposed to Giardia was lethal to *C. elegans*. Giardia or *E. coli* alone did not alter worm viability. 172 *E. coli* (HB101) genes, including many that are involved in stress response and metabolic pathways, were altered by co-incubation with Giardia (or with its excretory-secretory products) and/or *C. elegans*. *E. coli* genes that were positively upregulated included flagellar/adhesion genes, like *fljP* and *fljL*. Co-incubation with Giardia induced *E. coli* to colonize the gut of *C. elegans*, which untreated *E. coli* failed to achieve. Genes involved in hydrogen sulfide biosynthesis were decreased by exposure to Giardia, and indeed, deletion of the *cysB* gene in *E. coli*, a positive regulator for hydrogen sulfide biosynthesis genes, was sufficient to kill *C. elegans*, in the absence of Giardia. *E. coli* exposed to Giardia induced lethal paralysis in *C. elegans*, which could be similarly induced by human gut microbiota exposed to Giardia, or by Giardia-treated genetically inactivated *Citrobacter rodentium*, rendered non-virulent by mutation of the *espF* and *map* genes. The lethal effects required exposure of *C. elegans* to live bacteria. Additional experiments demonstrated that human gut microbiota rendered dysbiotic by exposure to Giardia are able to induce apoptosis, break down epithelial tight junctions, and translocate in human enterocyte monolayers. The findings suggest that Giardia may cause metabolic changes and induce virulence in non-pathogenic bacteria, and that the toxic consequence of these modifications can be observed in *C. elegans*. Exposure to Giardia is able to restore virulence in bacteria mutated into non-virulent states, and the effects is also lethal to *C. elegans*. Exposure to Giardia induces human gut microbiota to cause pathology in human enterocytes. We speculate that such activation of pathobionts may contribute to the development of post-infectious intestinal inflammatory disorders like IBS or flares in patients with IBD, long after the inciting enteropathogen has been eliminated. *C. elegans* offers intriguing opportunities to test human microbiota toxicity in a simple, high-throughput model system *in vivo*.

PERFORMANCE OF VELUM[®] ONE AGAINST PLANT PARASITIC NEMATODES AND EFFECT ON YIELD IN GRAPE PRODUCTION. Cabrera, A.J.¹, A. Kurokawa¹, A. Rodriguez², and S. Krueger¹. ¹Bayer CropScience LP, 2 TW Alexander Drive, Research Triangle Park, NC 27709. ²Department of Plant Sciences, California State University Fresno, 2415 East San Ramon Ave. Fresno, CA 93740.

Plant parasitic nematodes are an important pest in California grape production. Nematode infestations can result in poor vine growth, reduced yield and decrease the overall vineyard productivity. Methyl bromide has been phased out as a pre-plant soil fumigant in California perennial crops. Alternative pre-plant soil fumigants are highly restricted and regulated, some having township caps and buffer zones requirements. Currently there is a need for non-fumigant nematicides that can be used as post-planting treatments. Velum[®] One is a novel contact nematicide with fluopyram as active ingredient that has a different mode of action than traditional cholinesterase inhibitors. Fluopyram inhibits nematode mitochondrial respiration via inhibition of quinone dependent succinate reductase (also called Complex II). The objective of this investigation was to evaluate the performance of Velum[®] One to suppress a variety of important plant parasitic nematodes under field California conditions, and to evaluate its effect on grape yield. Efficacy trials in Flame grape vines conducted near Delano and then repeated

near Fresno showed that a single application of Velum[®] One through chemigation significantly ($p < 0.05$) reduced the number of citrus (*Tylenchulus semipenetrans*) and root-knot nematodes (*Meloidogyne arenaria*) compared to the untreated control. In a multi-year study from 2011 to 2014 in Thompson Seedless vines near Bowles, grape yield increased where Velum[®] One treatment was applied through chemigation twice per year. In particular, a yield significant difference was obtained in the third year of evaluation. In addition, there was a trend that the ring nematode (*Mesocriconema xenoplax*) population declined over time in the Velum[®] One treatment when compared to the untreated control. These field studies demonstrated the potential for Velum[®] One to be used as a post-planting tool for nematode management in grape production.

GENETIC VARIABILITY AND PHYLOGENETIC ANALYSES OF *NACOBBUS ABERRANS SENSU LATO* POPULATIONS BY MOLECULAR MARKERS. Cabrera-Hidalgo, A. de J.¹, N. Marbán-Mendoza¹, and E. Valadez-Moctezuma².
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Genetic variability of Mexican *Nacobbus aberrans* populations was detected by ISSR markers and some genes used in taxonomic studies. The populations of *N. aberrans* were isolated from different hosts in Guanajuato and Michoacan (Mexico). Sequence analysis of the 18S, Internal Transcribed Spacer (ITS) and Cytochrome Oxidase I (COI) regions was used in order to identify and estimate variability in the nematode populations studied. Several inter-simple sequence repeat (ISSR) markers were developed with four anchored primers for the comparative study of genetic variation. According to the ISSR analysis, 88.12% out of 150 bands were polymorphic. This technique grouped populations into two main clusters revealing a high level of genetic variability separating the Romita population from the others, with genetic similarity indices ranging from 0.44 to 0.82. Analysis based on partial sequences of the 18S and ITS regions indicated that our populations of nematodes corresponded to *N. aberrans*. However, the mitochondrial gene revealed significant levels of variation among the sequences analyzed, showing 16 variable sites. These results suggested that in the sampled areas there are at least two different biotypes of *N. aberrans*.

DIVERSITY OF *MELOIDOGYNE* SPP. FROM COFFEE AND MULTI-RESISTANT REACTION OF *COFFEA* SPP. TO ROOT-KNOT NEMATODES. Carneiro, R.M.D.G.¹, M.F.A. Santos¹, and V.R. Correa².
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Root-knot nematodes (RKN) are one of the main threats to coffee plantations in almost all producing regions. The identification of the main *Meloidogyne* species from coffee is now possible using esterase phenotypes and species-specific SCAR markers. During the last decade, extensive surveys and studies in South and Central America have shown a wide range of species, including the three major species, e.g. *M. exigua*, *M. paranaensis* and *M. incognita*. Other species such as *M. arabicida* and *M. izalcoensis* are restricted to Costa Rica and El Salvador, respectively. Genetic control of RKN constitutes an essential approach for integrated pest management strategy. Our studies in Brazil evaluated the multi-resistance of *Coffea canephora* genotypes (clones) to *Meloidogyne* spp. Sensitive and drought-tolerant coffee genotypes were used to infer their resistance using nematode reproduction factor. Clones 14 (drought-tolerant), CcK and CcR2 were the only genotypes highly resistant to three major species of *Meloidogyne*. ‘Clone 14’ (resistant) and ‘Clone 22’ (susceptible) were histologically studied upon infection by *M. incognita* race 3 and *M. paranaensis*. Four resistance mechanisms were observed in ‘Clone 14’: reduction of juvenile (J2) penetration inside the roots, early hypersensitive reaction (HR), late intense HR, and cell death around young females and giant cells were frequently observed. These results provide rational bases for future studies, including prospection, characterization and profiling expression of genomic loci involved in both drought tolerance and resistance to multiple RKN species. Diversity studies showed high intraspecific polymorphism for all *M. exigua* populations that reproduce by meiotic parthenogenesis. Two populations of *M. exigua* were of particular concern because they can reproduce on coffee cv. IAPAR 59 containing the Mex-1 resistance gene. Low molecular variability was observed in *M. incognita* and *M. paranaensis* populations from coffee which reproduce by mitotic parthenogenesis. No virulence related to different populations of these RKN species was observed in resistant coffees.

INVESTIGATION OF ROOT-KNOT NEMATODE MALE BEHAVIOR IN PLURONIC GEL. Čepulytė-Rakauskienė, R. and V.M. Williamson. Department of Plant Pathology, University of California, Davis, CA 95616.

Root-knot nematodes (RKN) infect over a thousand plant species and cause huge crop yield losses worldwide. With increasing restrictions on chemical use, alternative strategies to control or manage the negative impacts of RKN are urgently needed. RKN are sedentary endoparasites where females are immobilized once they establish a feeding site in the root. Some RKN species reproduce parthenogenetically; however, others such as *Meloidogyne hapla* can also reproduce sexually i.e., vermiform males must find sedentary females to fertilize. There is a huge information gap on what attracts plant parasitic

nematode males to females, but chemical signaling and sensing likely plays a significant role in this behavior. To address this hypothesis, we developed assays to assess *M. hapla* attraction and behavior using a thermo-reversible gel, Pluronic F-127 (PF127). PF127 is non-toxic and highly transparent. Stable chemical gradients can be formed in the gel. Both juveniles and males of RKN are able to move through the gel, facilitating determination of response to chemical signals. RKN males, females and egg masses were hand collected from 8-week-old nematode cultures propagated on tomato roots under greenhouse conditions. Males were suspended in PF127 gel and their attraction to females and to egg masses was assessed. Males were strongly attracted to females and egg masses as well as to exudates collected from females and egg masses. Previous chemical analyses of exudates from RKN juveniles revealed that these exudates contain ascarosides, a family of compounds that are glycosides of the sugar ascarylose with a fatty acid-derived side chain and that ascaroside #18 (ascr#18) is the most abundant member. Distinct ascarosides and blends regulate a range of social behaviors and developmental pathways of diverse nematode species. We found that synthetically produced ascr#18 (10 nM) attracted *M. hapla* males. Additional studies are underway to improve our understanding of RKN male behavior and its potential role in the life cycle of plant parasitic nematodes.

MENTHA SPICATA: A POTENTIAL LIVING MULCH FOR CONSERVATION AGRICULTURAL PRACTICES IN TROPICAL CLIMATES. Chan, K.D.¹, B. Sipes¹, K.H. Wang¹, and P.S. Leung². ¹Department of Plant and Environmental Protection Sciences, and ²Department of Natural Resources and Environmental Management, University of Hawaii at Manoa, 96822.

Mint (*Mentha spp.*) is used worldwide for a variety of consumption purposes. Mint has potential for use as a living mulch in smallholder tropical production as its runners allow establishment of a good ground cover while producing fine adventitious roots that are ideal for soil erosion prevention and protection of the soil structure. Mint roots uptake excess water while contributing to less water run-off and increasing soil moisture retention. In addition to these benefits of a living mulch, mint can add additional income when harvested. *Meloidogyne incognita*, *M. javanica* and *Rotylenchulus reniformis* are common plant-parasitic nematodes found in tropical climates. These nematodes reduce crop yield and quality in many vegetable crops. Consequently, susceptibility of a ground cover to these nematodes should be considered when designing conservation agricultural practices using living mulches. A greenhouse pot experiment was undertaken to determine the host status of spearmint and peppermint for these nematodes. Spearmint and peppermint plants were inoculated with *M. incognita*, *M. javanica* and *R. reniformis*. After 2 months, nematode eggs were collected and counted. Mint roots and shoots were weighed. The experiment was repeated once. Neither mint species was a host to these nematodes. The reproductive factor was less than 1 for each nematode on both plants. Fresh shoot weight in spearmint inoculated with *R. reniformis* was greater than the uninoculated plants. Fresh root weight of both mints inoculated with *R. reniformis* had a lower weight than the uninoculated plants. The added value of mint as a living mulch was determined in a field experiment with eggplant. Eggplant was intercropped with spearmint and was compared to that planted in bare ground. At 9 months after eggplant planting, eggplant yield in plots with spearmint was higher than eggplant in bare ground plots ($P < 0.05$). Using other Hawaii crops as a guide, along with primary and secondary data, the plots with eggplant intercropped with mint increased revenue for the farmer by more than 300%. Desirable traits of mint as a living ground cover, non-host to *M. incognita*, *M. javanica*, and *R. reniformis* compounded with mint's contribution of additional income can potentially benefit the smallholder farmer when used as a living mulch in a conservation agricultural production system.

NEMATODOS FITOPARASITOS DEL CULTIVO DE TOMATE (*LYCOPERSICUM ESCULENTUM* MILL.) DE TRES REGIONES DEL PERÚ. Checahuari-Jarata, S.¹, L.M. Israel¹, B.P. Rosario¹, Z.T. Noely¹, F.CH. Yeni¹, A.G. Marilia¹, M.C. Zheyla¹, y G.A. Sthewart¹. ¹Universidad Nacional del Altiplano, Puno. UNAP, PERU.

La producción de tomate en el Perú constituye una fuente importante para la economía del agricultor, sin embargo es afectada en su rendimiento por diferentes plagas como nematodos fitoparasitos, causando nodulaciones en la raíces, obstrucción en el paso de nutrientes, amarillamiento y marchitez de las hojas. El objetivo del presente trabajo es verificar la incidencia de diferentes géneros de nematodos fitoparásitos en el cultivo de tomate de tres regiones del Perú. Se recolectaron 95 muestras de suelo y de raíces de las regiones Puno, Tacna y Arequipa; se procesaron las muestras a través del método de fluctuación centrifuga en solución de sacarosa. Los géneros identificados fueron: *Helicotylenchus* spp., *Meloidogyne* spp., *Mesocriconema* spp., *Pratylenchus* spp., *Xiphinema* spp., *Tylenchus* spp y nematodos de vida libre (58.03; 46.31; 11.97; 7.29; 7.12; 1.21 y 82.89% respectivamente); cabe mencionar que las poblaciones de *Meloidogyne* fueron altas, precisando realizar un plan de control.

INCREASE IN VIRULENCE OF *HETERODERA GLYCINES* ON SOYBEAN OVER TIME IN THE PAST TWO DECADES IN MINNESOTA. Chen, S. University of Minnesota Southern Research and Outreach Center, 35838 120th Street, Waseca, MN 56093.

The soybean cyst nematode (SCN), *Heterodera glycines*, is a major biotic yield-limiting factor of soybean. Since it was first found in 1978, infestation of SCN in Minnesota gradually spread across the state and now has been found in most (>67) soybean-producing counties. SCN-resistant soybean cultivars have been used in Minnesota for more than two decades. The

SCN resistance of most cultivars was from the source of resistance PI 88788 and a small portion of cultivars had resistance from Peking. From 1997 to 2013, state-wide surveys were conducted four times to determine changes of SCN virulence phenotypes (HG Types) in Minnesota. The samples in the surveys in 1997-1998 and 2002 were randomly selected from the pool of samples submitted by soybean growers. The samples in 2007-2008 and 2013 were systematically taken from most soybean-growing counties based on soybean acreages. The reproduction potential measured as Female Index (FI) of the SCN populations was determined on the four race differential soybean lines (Pickett 71, Peking, PI 88788, PI 90763) plus PI 437654 in the first survey in 1997-1998 or the seven HG Type indicator lines (Peking, PI 88788, PI 90763, PI 437654, PI 209332, PI 89772, and PI 548316) in the other three surveys with Lee 74 as the susceptible control. There was limited increase of virulence of the nematode populations on the major sources of resistance PI 88788 and Peking from 1998 to 2002, while a dramatic increase of virulence was detected from 2002 to 2008 although there was limited further increase of the virulence from 2008 to 2013. In the 1997-1998 and 2013 samples, HG Type 0- was predominant and represented 85% and 81.1% of the populations, respectively. In 2007-2008 and 2013 samples, HG Type 2- that is virulent (FI > 10) to PI 88788 was predominant and represented 72.6% and 75% of the populations, respectively. HG Type 1- that is virulent to Peking also increased from 1.1% in 2002 to 12.1% in 2007-2008, and 22.9% in 2013. FI on PI 90763 and PI 89772 was highly correlated with FI on Peking, while FI on PI 209332 and PI 548316 was highly correlated with FI on PI 88788. Only one population collected in 1997 had FI more than 10 on PI 437654, and there was no increase of FI on it over the two decades. This study suggests that an extensive integrated approach including diversified sources of resistance, appropriate crop rotations, and other cultural and biological control methods are needed for a long-term effective management of the nematode.

POPULATION DIVERSITY OF SOYBEAN CYST NEMATODE IN NORTH DAKOTA FIELDS. Chowdhury, I.¹, G.P. Yan¹, A. Plaisance¹, B. Nelson¹, S. Markell¹, T.C. Helms², and A. Upadhaya¹. ¹North Dakota State University, Department of Plant Pathology, Fargo, ND 58108, ²NDSU, Department of Plant Sciences, Fargo, ND 58108.

Soybean cyst nematode (*Heterodera glycine*; SCN) is responsible for the greatest annual yield loss, more than \$1 billion, among all pathogens of soybean in the United States. To assess the prevalence of SCN in North Dakota, soil samples were collected in 2015 from soybean fields or fields with a history of SCN across 14 counties. SCN eggs were extracted and quantified. Out of 155 fields surveyed, 87 were infested with SCN. The egg population densities in these fields ranged from 25 to 21,540/100 cm³ of soil with an average density of 890 eggs/100 cm³ soil. To characterize the genetic diversity in these populations, HG type bioassays were performed. The assay was conducted with seven soybean plant introduction lines (PI 548402, PI 88788, PI 90763, PI 437654, PI 209332, PI 89772 and PI 548316) used as test lines and two local cultivars (Sheyenne and Barnes) as susceptible checks. Inoculated plants were grown in a growth chamber for approximately 30 days at 27 °C. To date, 27 of these SCN populations were assayed using naturally infested soil. In order to conduct the HG type bioassays on nine field samples with low numbers of eggs, inoculum was first increased by incubating the populations on susceptible cultivars for 90 days under the same conditions. Then, HG type bioassays were performed on these populations by infesting autoclaved river sand with 2000 eggs for each plant. Both naturally infested soil and artificially infested soil were used for the first iteration of HG type testing. To confirm new HG type results from the first iteration of testing, some experiments were repeated a second and third time using artificially infested river sand with 2000 eggs. Seventy percent of the first iteration of these experiments had greater than 100 SCN white females on susceptible checks indicating that the experimental conditions were optimal for SCN reproduction. Among the successful experiments, the most common HG types were HG type 0 (frequency rate: 32%) and HG type 7 (20%). Other HG types included 2.5.7 (8%), 2.7 (8%), 2.5 (8%) and 5 (8%). Through the second and third iteration of the experiments we were able to confirm SCN populations from ND fields had HG type designation of 7 and 2.5.7. Only HG type 0 was previously reported in ND. The new HG types detected give us a greater insight into SCN population diversity in ND. With such information, farmers and researchers in ND will be better equipped to combat soybean yield losses due to SCN. More fields in the areas with the new virulent types will be surveyed and assayed in 2016 to monitor virulence changing of SCN populations in ND.

MORFOLOGÍA, BIOLOGÍA Y ECOLOGÍA COMO APOYO A LA TAXONOMÍA CLÁSICA DE NEMATODOS. Cid del Prado Vera, I. Laboratorio de Nematología, Programa de Fitopatología, Colegio de Postgraduados. 56230 Texcoco, Estado de México.

La taxonomía de nematodos y otros organismos ha sido una creación del hombre, para identificarlos, conocer su diversidad, su filogenia y sus procesos evolutivos. Tradicionalmente se ha basado en la morfología y en la morfometría de los especímenes, y ha generado la descripción de aproximadamente 25 mil especies registradas hasta ahora del Phylum Nematoda, de un número estimado existente de 500 mil, el cuarto filo del reino animal en número de especies. Muchas de las descripciones de nuevas especies en el pasado se ha realizado tras el estudio de muy pocos individuos y teniendo en cuenta solamente algunas características morfológicas visibles a través del microscopio óptico. Con frecuencia, otros rasgos morfológicos han pasado desapercibidos y se han omitido. El resultado ha sido que muchas especies permanecen en la actualidad pobremente descritas, se consideran sinónimas de otras o no son aceptadas como especies válidas puesto que la

información disponible sobre ellas es incompleta y/o errónea. A menudo, esto ha traído consigo confusión e inseguridad a la hora de presentar diagnósticos fiables, tanto a nivel de género como de especie. En las últimas décadas, sin embargo, la situación ha mejorado considerablemente debido al desarrollo y la aplicación de nuevas técnicas de estudio. Así, la información proporcionada por la microscopía electrónica, tanto de transmisión como de barrido, la inclusión de nuevos aspectos biológicos y ecológicos en el estudio de la diversidad, y, muy especialmente, las técnicas moleculares y de análisis estadístico han contribuido a una mejor caracterización y diagnóstico de las especies y ha favorecido y fortalecido la propuesta de nuevos taxones de nematodos. Es necesario, no obstante, más trabajo en el estudio de la variación intraespecífica y geográfica de las especies, con una nueva generación de taxónomos especializados que continúen la labor de completar el conocimiento de la diversidad nematológica.

NACOBBUS ABERRANS IN HORTICULTURAL CROPS AND ITS MANAGEMENT IN MÉXICO. Cid del Prado Vera, I. Laboratorio de Nematología, Programa de Fitopatología, Colegio de Postgraduados. 56230 Texcoco Estado de México.

The false root-knot nematode, *Nacobbus aberrans*, is distributed in the central part of Mexico from the Coahuila State to the south of Oaxaca State, parasitising important crops, including beans, tomato, chile, sugarbeet and other crops, as well as weeds. Damage is severe and crop losses are up to 50%. Studies on morphology and morphometrics of the nematode were conducted in 1996 with the objective of determining whether Mexican populations constitute a single species. Differences between populations were not significant. Biological studies revealed that the third and fourth juvenile stages enter dormancy and form the primary inoculum in infested soils. A field study in 1995 evaluated genetic resistance in varieties of bean; the native varieties Amarillo Calpan and Negro San Luis and the genetically improved varieties Bayo Mecentral and Rio Grande were resistant. In 2000 and 2001, soil amendment experiments tested incorporation of *Ricinus communis* and *Brassica oleracea* into infested soil. Gall indices were reduced but there was some phytotoxicity. Since 2009 we have been testing and improving biofumigation for nematode control in tomato and bell pepper in greenhouses. The process includes application of crucifer residues, chicken or cow manure, at levels of 1 ton/1000 am², and the fungus *Pochonia clamydosporea* at 1.9x10⁷ chlamydospores one month after the biofumigation. The trophic structure of the nematode fauna changes, population levels of *Nacobbus aberrans* decrease, and gall indices are reduced from 8-9 to 3. These results are very encouraging and suggest opportunities for further research to optimize application rates of organic amendments testing plant residues with different nematicidal properties, and combination with other control methods.

BIOLOGICAL CONTROL OF THE PALE CYST NEMATODE GLOBODERA PALLIDA WITH TRICHODERMA HARZIANUM. Contina, J.B.¹, G.R. Knudsen¹, and L.M. Dandurand². ¹Soil and Land Resources Division; ²Plant Science Division, University of Idaho, Moscow, ID 83844.

Trichoderma harzianum strain ThzID1-M3, isolated in the state of Idaho and transformed to express green fluorescent protein (GFP), was evaluated as a potential biocontrol agent against the pale cyst nematode (PCN) *Globodera pallida* in potato (*Solanum tuberosum* L.). *G. pallida* is a quarantine pest in Idaho, is globally regulated, and represents a major threat to the Idaho potato industry. The objectives of this study were to evaluate ThzID1-M3 as a biocontrol agent to reduce *G. pallida* infections in potato roots, to assess its ability to colonize nematode cysts, and its ability to proliferate in soil and on roots. ThzID1-M3 was maintained on *Trichoderma* selective media, and was allowed to grow on sterile oat kernels at 25°C for 20 days. *G. pallida* cysts were surface-sterilized with sodium hypochlorite, rinsed thoroughly, hydrated for 3 days and placed in nylon mesh bags. Greenhouse experiments were conducted for 45 and 75 days, respectively. Potato tubers cv 'Russet Burbank' were used, and a sterile mixture of 2:1 sand: silt loam was used. Experimental treatments were as follows: control, ThzID1-M3 (only), PCN (only), and ThzID1-M3 applied together with PCN. Treatments were replicated five times in a completely randomized block design. DNA extraction and PCR analysis of cysts, roots and soil samples were done using standardized molecular kits. Results in experiment 1 showed significant reduction in nematode infections in potato roots ($t < 0.05$). In experiment 2, significant reduction of *G. pallida* cysts in the soil was observed ($t < 0.05$). However, no significant changes were detected in the number of eggs/cyst, eggs/g of soil, and reproduction factor ($t > 0.05$). No significant effects were detected in biomass and root weight ($P > 0.05$). PCR analysis and dilution plating showed that ThzID1-M3 was able to colonize *G. pallida* cysts, potato roots, and proliferate in soil. In a third experiment, transparent rhizosphere chambers were used to observe fungus/nematode interactions on root surfaces. The introduced nematoparasitic fungus *T. harzianum* ThzID1-M3 shows potential for biocontrol of *G. pallida*. Further research will shed light on interactions between ThzID1-M3 and *G. pallida*, and hopefully lead to implementation of biocontrol strategies.

NEW NEMATICIDES FOR GOLF COURSE TURFGRASSES. Crow, W.T. Entomology and Nematology Dept., University of Florida, Gainesville, FL 32611.

Several new nematicides have been launched, or are expected to be launched, for use on golf course turfgrasses in the USA in 2016. The active ingredients in these new nematicides include abamectin, fluensulfone, and fluopyram. Each of these active ingredients is effective, but each has relative strengths and weaknesses, particularly depending on the type of nematode targeted. The biology, mode of feeding, and seasonal population dynamics of the targeted nematode greatly impact nematicide efficacy

and optimal application timing for each active ingredient. The University of Florida has years of turfgrass field data evaluating the efficacy of these active ingredients against the three most problematic nematode genera in Florida golf courses; sting (*Belonolaimus* spp.), root-knot (*Meloidogyne* spp.), and lance (*Hoplolaimus* spp.) nematodes. These data, and explanations for the relative efficacy of each nematicide against these three nematode genera, will be summarized. Current recommendations for use of abamectin, fluensulfone, and fluopyram for nematode management on golf course turf in Florida will be discussed.

INTEGRATED MANAGEMENT STRATEGIES FOR *HETERODERA SCHACHTII* IN GERMAN SUGAR BEET CROPPING SYSTEMS, Daub, M. Julius Kuehn-Institute, Dürener Str. 71, D 50189 Elsdorf, Germany.

The white sugar beet cyst nematode (BCN) *H. schachtii* has a 150-year history of causing economic damage in the main sugar beet growing regions in Germany. Without nematode management population densities of *H. schachtii* can easily exceed 5000 E+J/100 ml soil resulting in 30-50% yield loss. Nematicides are no longer available since 2000, and registrations of new nematicides are still rare due to strict EU requirements. Therefore, crop rotation in combination with targeted use of resistant catch crops are established standard tools used to maintain BCN population densities below the damage threshold level of 500 eggs and juveniles (E+J)/100 ml soil. Since registration of the first nematode tolerant cultivar in 2004 use of this cultivar type continuously increased up to 80% and more in BCN infested areas. Tolerant cultivars show lower multiplication rates of BCN than susceptible cultivars which most likely derives from a polygenic resistance background associated with the tolerance factors of the wild beet *Beta maritima* used for breeding. However, very little is known about the stability of this resistance background and if continuous cropping of tolerant cultivars could select for higher virulence in BCN populations. Despite the overall higher yield potential of tolerant cultivars, nematode damage of up to 20% at BCN densities exceeding 2000-4000 E+J/100 ml have been measured in field trials. This yield reduction is not visible to growers as it is masked by the physiological tolerance of this cultivar type even during dry and hot summer periods. Consequently, growers tend to neglect standard integrated nematode management practices expecting to achieve maximum yield by just growing tolerant cultivars. One of the future challenges for BCN management strategies will be to draw attention of growers to non fully exploited yield potentials rather than visible damage reactions of sugar beets. With opening of the EU sugar market in 2017, the German sugar industry becomes even more dependent on high productivity as land in traditional growing areas is very limited. Addressing these future challenges integrated nematode management will depend on a sufficient basic and applied nematological research program to protect sustainability of sugar production in intensive cropping systems.

INCREASED SIZE OF COTTON ROOT SYSTEM DOES NOT IMPART TOLERANCE TO *MELOIDOGYNE INCOGNITA*. Davis, R.F.¹, P.W. Chee² and E.L. Lubbers². ¹USDA-ARS, Crop Protection and Management Research Unit, Tifton, GA, ²University of Georgia, Crop and Soil Science Dept., Tifton, GA.

Plant tolerance or intolerance to parasitic nematodes represent a spectrum describing the degree of damage inflicted by the nematode on the host plant. Tolerance is typically measured in terms of yield suppression. Instances of plant tolerance to nematodes have been documented in some crops, including cotton, but the mechanisms of tolerance are not understood. We hypothesized that cotton plants with larger, faster-growing root systems would be more tolerant to *Meloidogyne incognita* because a larger root system could result in fewer nematodes per gram of root thereby reducing the parasitic load on the plant. Eleven cotton germplasm lines were selected because previous research showed their root systems (in the absence of nematodes) differed in weight, taproot length, and number of lateral branches. A field study was conducted for two years to determine whether those root system attributes were related to tolerance of the plants to *M. incognita*. Cotton lines were grown in a split-plot design with fumigated and nonfumigated subplots so that percentage yield loss could be calculated for each genotype. Analyses included variables that were standardized for each genotype as a percentage of the maximum (e.g., root length as a percentage of the genotype with the greatest length). Root data from previous research on these genotypes along with yield data from the current test were used to evaluate potential relationships among variables. Regression analyses showed that percentage yield loss was not related to percentage maximum weight or percentage maximum number of lateral branches. However, percentage yield loss increased as the percentage of maximum length increased, which is the inverse of what our hypothesis predicted. Perhaps nematode parasitism inhibits root elongation in seedlings and young plants which prevents plants from reaching their full rooting depth thereby resulting in a relatively greater effect on plants with the greatest potential for root length. Therefore, we conclude that cotton plants with larger root systems are not more tolerant to *M. incognita*.

ASSOCIATION BETWEEN SOIL FUNGI AND PRATYLENCHUS BRACHYURUS IN SOIL OF CERRADO BIOME. De Oliveira, C.J.¹, A.A. Chaibub⁴, F.J. Gonçalves³, R.A. Teixeira¹, K.C.L. Sousa², B.L. Mendes², S. Agnonsou², M.R. Rocha¹, and L.G. Araújo². ¹Nematology Laboratory, Federal University of Goiás, Goiânia, Brazil. ²Genetics of Microorganism Laboratory, Federal University of Goiás, Goiânia, Brazil. ³Brazilian Agricultural Research Corporation (Embrapa), Santo Antonio de Goiás, Brazil. ⁴University of Brasilia, Brasilia, Brazil.

Nematodes cause losses of 8% to 10% of soybean yield, in Brazil, according to The Brazilian Agricultural Research Corporation (Embrapa). Mato Grosso State, the largest grain-producer state in Brazil, can lose around two million tons of soybean yield due to nematode damage. Nematode control has been very challenging, because most of chemicals currently

available on the market have a negative impact on the environment and is not always efficient. Biological control is a promising measure to reduce nematode population in the field. The objective of this study was to quantify the number of fungal colony-forming units (CFU) ml⁻¹ from soil and to identify fungi from soil of a cerrado biome infested with *Pratylenchus brachyurus*. We employed a factorial design, four replications and four treatments; T1: centrifuged nematodes (collected from green house soil) in a saccharose solution; T2: 10g of non autoclaved soil from cerrado, infected by *P. brachyurus*; T3: 10g of soil from the green house, infested with *P. brachyurus*; T4: 10g of soil from cerrado not infested with *P. brachyurus*. Approximately 10.000 nematodes ml⁻¹ were used for a serial dilution, from 10⁻¹ to 10⁻⁵, in the treatments (T1). For the other treatments (T2, T3 and T4), 10g of soil was added in 90 mL of autoclaved water, followed by a serial dilution, from 10⁻¹ to 10⁻⁵ for the treatments. One ml of all dilutions, from each treatment was transferred to Petri plates containing PDA medium. After 48 h of incubation, all treatments were evaluated by counting CFU ml⁻¹ of soil. There was none CFU of fungous grown in the treatment T4. T1, T2 and T3 presented CFU, which were counted and identified, after 108 h of incubation. Data were analyzed by two ways ANOVA, using 9,999 randomized (5%). There was interaction between the treatment and the dilution (p= 0.0019). The dilutions 10⁻¹, 10⁻² and 10⁻³ from the T1 were those dilutions that were found the higher CFU ml⁻¹ of soil and nematode. The number of CFU ml⁻¹ was higher in the treatments T1, T2 and T3. According to Barnett and Hunter (2003), it was identified as *Trichoderma* sp., *Penicillium* sp., *Aspergillus* sp. and *Fusarium* sp.. The most abundant was *Trichoderma* sp., indicating that it can be used as biological agent against *Pratylenchus brachyurus*.

PROPOSAL FOR GENOME AND TRANSCRIPTOME ANNOUNCEMENT ARTICLES IN THE *JOURNAL OF NEMATOLOGY*. **Denver, D.R.¹, E.J. Ragsdale², W.K. Thomas³, and I.A. Zasada⁴**. ¹Department of Integrative Biology, Oregon State University, Corvallis, OR 97331, USA; ²Department of Biology, Indiana University, Bloomington, IN 47405, USA; ³Hubbard Center for Genome Studies, University of New Hampshire, Durham, NH 08324, USA; ⁴USDA-ARS Horticultural Crops Research Laboratory, Corvallis, OR 97330, USA.

We propose that the *Journal of Nematology* offer scientists the opportunity to publish Genome Announcement and Transcriptome Announcement articles. These are very short (~500 words), peer-reviewed articles whose main purpose is to announce the initial sequencing, assembly, and public database deposition of data associated with a nematode genome or transcriptome. Animal genomes and transcriptomes can now be sequenced very quickly and at very low cost. Numerous (certainly many dozens, perhaps up to 100) nematode genomes and transcriptomes have been sequenced and assembled by an increasingly large number and diversity of nematologists and genome-oriented biologists. If broadly available to the scientific community, these resources would have the potential to lead to important new insights into diverse areas of nematode biology including phylogenetics and taxonomy, genome structure and evolution, and effector gene characterization. Few of these genomic and transcriptomic resources, however, have been deposited into public sequence databases. Why? One obvious reason is that many of those scientists who have invested resources and time into generating the 'omic resource want to make sure that they are credited for the work – a high profile, peer-reviewed paper is generally the desired outcome, with the necessary accompanying submission to a public sequence database. However, it is a common story that such efforts take a very long time (many months to years) for a variety of reasons that include challenging bioinformatic analyses and the coordination of large collaborative groups that often underlie such efforts. Genome and Transcriptome Announcement articles would be mutually beneficial to the broader scientific community, the scientists collecting 'omic data, and to the *Journal of Nematology*. For the scientific community, these short articles would provide a mechanism for genome and transcriptome resources to be submitted to public databases faster. For the scientist, they would provide a simple and fast path for publishing basic 'omic information in a fashion that results in a peer-reviewed and citable product. For the *Journal of Nematology*, they would add a new and innovative service to the worldwide community of nematologists and genome biologists.

THE EFFICACY OF NEMATICIDE TREATED SOYBEAN VARIETIES TO REDUCE IMPACT OF SOUTHERN ROOT-KNOT NEMATODE. **Dodge, D. and K. Lawrence**. 209 Rouse Life Sciences, Auburn University, Auburn, AL 36849.

Southern root-knot nematode or *Meloidogyne incognita*, (RKN) is a common plant parasitic nematode in the Southeastern United States that hampers plant development and reduces yield. Resistant varieties and nematicides are two methods of control and yield loss prevention for these plant parasitic nematodes. This study screened susceptible, moderate resistant, and resistant varieties in the presence and absence of the nematicide Abamectin (Avicta) in order to determine the efficacy of the nematicide seed treatment to prevent biomass and yield reduction caused by this phytopathogenic nematode. Ten soybean varieties were evaluated: one root-knot (RKN) susceptible, four moderate resistant, and five resistant varieties. The experiment used three factor tests, each having replicates of all varieties. The test groups were: Control (no RKN, no nematicide treatment), Variety (RKN inoculated, no nematicide treatment), and Nematicide (RKN inoculated, nematicide treated seed). Nematicide treated seeds received 0.15 mg Abamectin per seed. Greenhouse trials were conducted in 150cc cone-tainers in a RCBD with 5 replicates per treatment. Treatments to include RKN were inoculated with 2,000 *M. incognita* eggs at planting. Plant height, fresh shoot and root weights were recorded at 45 days after planting. Greenhouse trials were repeated and data

analyzed in SAS 9.4 by Tukey's ($P \leq 0.05$), comparing means across varieties and tests. The nematicide seed treatment Abamectin increased plant biomass by 5% on average ($P \leq 0.05$) in the presence of *M. incognita*. Root fresh weight was also increased 17% with the nematicide application. Abamectin decreased *M. incognita* eggs per gram of root by 77% and this nematicide treatment significantly reduced nematode egg densities across all varieties. Additionally, Abamectin increased biomass in resistant, moderately resistant and susceptible varieties similarly. Abamectin decreased eggs per gram of root in the susceptible variety by 84% (3,240 eggs per gram to 498), 75% (1,764 eggs per gram to 339 on average) in the moderate resistant varieties, and by 77% (860 to 179 on average) in resistant varieties on average. Additionally, Abamectin increased plant biomass by 10%, 1%, and 5% on average in susceptible, moderately resistant, and resistant varieties respectively. This test will be conducted in field trials in the 2016 season to determine the damage potential of the RKN by yield reductions and if the increase in plant biomass observed in the greenhouse translates into increased yield in varieties treated with a nematicide.

CHARACTERIZATION OF EFFECTOR GENES FROM *GLOBODERA PALLIDA* IN RESISTENT AND SUSCEPTIBLE POTATO PLANTS. Duarte, A. and L.M. Dandurand. Plant, Soil and Entomological Sciences Department, University of Idaho, 875 Perimeter Drive MS 2339, Moscow, ID 83844-2339.

The potato cyst nematode (PCN), *Globodera pallida* was first detected in the United States in Idaho in April 2006. PCN is one of the most economically important pests of potato, causing in excess of 80% yield loss in infested fields. The introduction and potential spread of potato cyst nematode has serious implications for U.S. potato production and export. Eradication efforts using fumigation are becoming increasingly challenging, due to regulatory pressures that will make strategies relying primarily on soil fumigation even more difficult. Currently, there are no commercially acceptable potato varieties in the U.S. with *Globodera* resistance that could be safely planted into deregulated fields. Nematode secretions and proteins from the surface coat are likely to be the first signals perceived by the plant. These molecules, known as effectors, are known to suppress the host defense response, facilitate the migration, and induce the feeding structure – the syncytium, essential for their development and reproduction. Understanding plant response to effector proteins can assist in the development of resistance. The purpose of this study was to determine key genes differentially expressed in different *G. pallida* life stages in susceptible and resistant potato genotypes. The effector genes calreticulin (*Gp-crt-1*), fatty-acid and retinol protein (*Gp-far-1*), protein rbp-1 (*Gp-rbp-1*), and superoxide dismutase (*Gp-sod*) genes were isolated and cloned from the Idaho population of *G. pallida*. Preliminary studies revealed that genes are expressed in all the developmental stages tested and has higher levels of expression in the juveniles (J2). Resistant and susceptible potato roots will be infected with J2 from *G. pallida* and the nematodes will be collected at differential time points (24h, 7, 14 and 21 days after infection). The characterization of these effector genes from *G. pallida* will lead to further understanding of the infection process.

BIOLOGICAL PATHOGEN OF *HETERODERA GLYCINES*, *ROTYLENCHULUS RENIFORMIS*, AND *MELOIDOGYNE INCOGNITA*. Dyer, D.¹, N. Xiang¹, K.S. Lawrence¹. ¹Dept. of Entomology and Plant Pathology, Auburn University, AL 36849.

Heterodera glycines and *Rotylenchulus reniformis* cultures in our greenhouse have a fungus colonizing the body of the nematode juveniles and forming sporangia inside the nematodes bodies. The sporangium produces zoospores and forms germination tubes to release the zoospores outside the cuticle of the nematodes. The morphological characteristics indicated that this fungus is a *Catenaria* sp. The objectives of this study were to determine the best isolation medium, the optimum fungal growth temperature, and to define the infection rates on the three nematodes. In all tests treatments were arranged in a RCBD and replicated 5 times and were assessed for fungal growth after 7 days. Data was analyzed with SAS 9.4 using PROC GLIMMIX and LS-means compared using Tukey-Kramer method with significant level of $\alpha \leq 0.05$. An individual *H. glycines* or *R. reniformis* vermiform nematode colonized with *Catenaria* sp. was placed on either 4% BEA (Beef Extract Agar), PDA (Potato Dextrose Agar), PCA (Potato Carrot Agar), OA (Oatmeal Agar), or CMA (Corn Meal Agar) and allowed to grow for 7 days and assessed for fungal growth. The results indicated that 4% BEA was the only media that support growth of the *Catenaria* sp. fungus from either of the nematode genera. Isolates of the *Catenaria* sp. were transferred to new 4% BEA plates and incubated at temperatures of 10, 20, 25, 30, 35, and 40 °C for 15 days. The optimum growth temperature for the isolates was found to range from 25 to 35 °C ($P \leq 0.05$). No fungal growth was observed at 10 or 40 °C. *H. glycines*, *R. reniformis*, and *M. incognita* juveniles and eggs, both live and dead were placed in different wells of the 96-well plates. One infective nematode was added to each well to observe the infection rates over a 20-day period. *M. incognita* infection rates for dead juveniles and dead eggs were 100% and 75% respectively, which were significantly higher than live juveniles which exhibited an infection rate of 3% ($P \leq 0.05$). The infection rate for live *M. incognita* eggs was 50% with no significant difference from living juveniles or dead eggs or juvenile nematode colonization. For *H. glycines* and *R. reniformis*, infection rates of the dead juveniles were as high as 100% at 20 days. No infection was found on the live *H. glycines* and *R. reniformis*. Future work will include greenhouse testing to assess the biological control ability of this *Catenaria* sp. on *H. glycines*, *R. reniformis*, and *M. incognita*.

MOLECULAR CHARACTERIZATION OF HETEROGENEOUS *PASTEURIA* SPP. SPORES WITHIN A SINGLE *HETERODERA GLYCINES* CYST. **Dyrdahl-Young, R.¹, R.M. Giblin-Davis², W.L. Nicholson³, L.W. Duncan⁴, S. Joseph¹, and T.M. Mengistu¹.** ¹Department of Entomology and Nematology, University of Florida, Gainesville, FL 32611, ² Entomology and Nematology Department, University of Florida, Fort Lauderdale Research and Education Center, Davie, FL 33314, ³Microbiology and Cell Science, University of Florida, Kennedy Space Center, Titusville, FL 32899, ⁴Department of Entomology and Nematology, Citrus Research and Education Center, University of Florida, Lake Alfred, FL 33850.

Pasteuria is a genus of obligate endoparasite, spore-forming bacteria with specific isolates antagonistic towards different genera of plant-parasitic nematodes. *Pasteuria nishizawae*, which parasitizes *Heterodera glycines* was first reported in North America in 1994. However, poor understanding of basic biology about lifecycle, ideal soil conditions for sporulation, and host preferences of the bacterium make it difficult to culture sufficient endospores for research purposes. Establishment of homogenous spore populations is an essential first step to efforts to optimize conditions for endospore augmentation. Previous reports found heterogeneity in *P. penetrans* genotypes within a single nematode. In order to elucidate the potential diversity of spore lines, an 800 bp fragment of the 16S rRNA gene with a 200 bp highly variable region was amplified from endospores. This region is reportedly able to segregate *Pasteuria penetrans* on an intrapopulation level. Spores from ten different *H. glycines* cysts were isolated and the genomic DNA was extracted from each sample. The variable region of 16srRNA was amplified and cloned from each sample. Ten clones from each of the ten samples were sequenced. The presence of multiple genotypes from within a single nematode will be discussed.

PROJECT NEMATODA: A COLLECTION OF ORIGINAL SPECIES DESCRIPTION OF EVERY NEMATODE. **Eisenback, J.D.** Department of Plant Pathology, Physiology and Weed Science, Virginia Tech, Blacksburg, VA 24060.

A recent study concerning specimens in natural history museums reported that half are wrongly labeled, but who is to blame and what are the solutions? Perhaps the paucity of taxonomists and the lack of man-hours devoted to identifying organisms versus the overwhelming numbers of specimens that need to be described and labeled are the reason for this quandry. In the last 250 years more than 25,000 species of nematodes have been described, an average of 100 descriptions per year. Furthermore, descriptions are published in many different journals in numerous languages from all around the world. Each researcher has to collect these species descriptions even though not all libraries subscribe to every journal and some are not open access, yet all of them are absolutely necessary to identify nematodes to species or to compile monographs or other taxonomic aids. Theoretically, type specimens are the backbone of nematode taxonomy and descriptions are linked to these types. Ideally, type specimens should be consulted whenever an unknown is trying to be identified, but permanent type specimens are not very “permanent” and are generally too valuable to be lent to most requesters. Travelling to the museums with types is an option, but can be expensive, time consuming, and may not be extremely productive. Often, the drawings, photographs, and textural description substitute for the “type”. One possible solution to this dilemma is to make resources that are necessary for taxonomy easier to access and more widely available. Project Nematoda is an attempt to collect the physical description of every species of nematode that has ever been described. The Project uses the name in the original publication and arranges them alphabetically by the genus name. Currently more than 15,000 species descriptions have been collected and easy access to these resources will be available on the internet to everyone with a computer and a connection.

EVALUACIÓN A CAMPO DEL USO DE NEMATODOS ENTOMOPATÓGENOS EN EL CONTROL DE *LOBIOPA INSULARIS* (COLEOPTERA: NITIDULIDAE) EN CULTIVOS DE FRUTILLA DE LA REGIÓN HORTÍCOLA PLATENSE, BUENOS AIRES, ARGENTINA. **Eliceche, D.P., A. Salas, J.M. Rusconi, and M.F. Achinelly.** CEPAVE, Facultad de Ciencias Naturales y Museo, (UNLP), CONICET. Argentina.

Lobiopa insularis es una de las principales plagas en cultivos de frutilla en la región hortícola platense, causando daños directos en los frutos debido a la alimentación de larvas y adultos, e indirectos por la colonización de microorganismos que aprovechan dicho daño, reduciendo el uso para consumo y/o comercialización. El sitio de estudio, Colonia Urquiza (La Plata, Buenos Aires), comprende una zona hortícola con un manejo orgánico de los cultivos. *Lobiopa insularis* representa un problema para los horticultores de la región debido a la falta de enemigos naturales. El control se ejerce principalmente mediante el manejo cultural, a través de la cosecha de los frutos antes de su maduración, evitando así atraer al coleóptero. Para su comercialización, el fruto debe estar en un estado de madurez apropiado según el color, contenido de azúcares y consistencia. Teniendo en cuenta el índice de madurez establecido por el SENASA (75% del fruto color rojo), y que la frutilla es una fruta no climatérica, la cosecha temprana contribuye al manejo del coleóptero, aunque puede disminuir la calidad del fruto comercializable. La aparición del coleóptero se da cuando las frutillas se encuentran maduras y/o fermentadas, por tanto la cosecha tardía representaría un problema para el horticultor. Como alternativa al manejo cultural se propuso evaluar a campo la potencialidad del nematodo entomopatógeno *Heterorhabditis bacteriophora* aislado en el sitio de estudio, como agente de control de *L. insularis*. La susceptibilidad del coleóptero al nematodo ha sido previamente evaluada en condiciones de laboratorio, arrojando resultados satisfactorios. Se realizaron liberaciones aumentativas de juveniles infectivos (JI) del nematodo, por medio de aplicaciones de suspensiones acuosas cada 5 pasos, la dosis utilizada fue de 10.000 JI en 3 ml de

agua. La unidad de estudio fue una parcela conformada por 7 camellones con dos hileras de plantas cada uno. El cultivo de frutilla se encontraba alternado a intervalos irregulares con plantas de tomate, berenjena, cebolla de verdeo y malezas, irrigado por sistema de riego y manejo agroecológico del mismo. Se utilizaron dos camellones como puntos de aplicación y dos como control. El monitoreo de la plaga se realizó cada 15 días, a partir de la estimación del daño en el fruto, cuyos porcentajes variaron entre 0-10%. Una vez abandonada la cosecha, se realizaron nuevos monitoreos en los cuales se registró un daño promedio en los controles del 46%, mientras que en los camellones aplicados fue del 23%. A partir de estos datos preliminares podemos inferir que las diferencias entre los tratamientos son significativas, por tanto el uso del nematodo como agente de control de *L. insularis* podría representar una estrategia de control alternativa al manejo cultural, garantizando la producción de frutos con las características demandadas por el mercado.

GENOME SCANS ON EXPERIMENTALLY EVOLVED *GLOBODERA PALLIDA* POPULATIONS TO IDENTIFY MOLECULAR BASIS OF ADAPTATION TO *GPAV_{VRN}* POTATO RESISTANCE. **Eoche-Bosy, D.¹, M. Esquibet¹, S. Fournet¹, M. Gautier^{2,3}, F. Legeai^{1,4}, A. Bretaudeau^{1,4}, E. Grenier¹, and J. Montarry¹.** ¹UMR IGEPP, INRA, 35653 Le Rheu, France, ²UMR CBGP, INRA, IRD, Cirad, Montpellier SupAgro, 34988 Montpellier-sur-Lez, France, ³Institut de Biologie Computationnelle, 34095 Montpellier, France, ⁴IRISA, INRIA, 35042 Rennes, France.

In the current agronomical context of reduced use of pesticides, deciphering the genetic bases of pathogen adaptation to plant resistances is of major importance as it could help us to improve durability of these resistances. *Globodera pallida* is a major pest of potato and for which the promising resistance factor, QTL *GpaV_{VRN}*, has been identified in *Solanum vernei* and introduced into several new resistant cultivars in Europe. However, a previous study having employed an experimental evolution protocol, in which *G. pallida* lineages evolved on resistant or sensible potato genotypes, showed that *G. pallida* was able to overcome the resistance from *S. vernei*. The aim of the present study was to investigate the genomic regions involved in the resistance breakdown using a genome scan approach on the lineages resulting from the experimental evolution. A first low throughput genome scan was performed using 202 microsatellite markers distributed along the genome, using three different tests of neutrality based on genetic differentiation and heterozygosity. We identified eight outlier loci, several of which were found by multiple outlier detection methods and/or in two independent adapted lineages, indicative of genomic regions putatively involved in the resistance breakdown where several effectors of interest were identified. We also showed that the same adaptive genetic pathways seem to be involved in overcoming potato genotypes that harbored the same resistant QTL but differed in their genetic background. These results have validated the feasibility of a genome scan approach on biological material coming from a short term experimental evolution and led us to target more precisely the genomic regions involved in the adaptation. Thus a second high-throughput genome scan was performed using NGS data obtained through a whole genome resequencing of pools of individuals (Pool-Seq) that belonged to some of the lineages coming from the experimental evolution. 1.6 million SNPs are currently being used for a genome scan performed with the BAYPASS program. Identified outlier loci will allow us to target more precisely the best candidate genes involved in the adaptation and will also provide a molecular tool to follow virulence allele frequencies within wild *G. pallida* populations, which would be useful in order to conceive efficient strategies for maximizing the durability of potato resistance.

THE EMERGENCE OF *MELOIDOGYNE HAPLANARIA* IN FLORIDA, AND THE EFFECT OF INITIAL DENSITIES POPULATIONS ON TOMATO. **Espinoza-Lozano, L.^{1,3}, S. Joseph¹, W. Crow¹, L. Duncan², J. Noling², and T. Mekete¹.** ¹Department of Entomology and Nematology, University of Florida, Gainesville, FL, 32608, ²Department of Entomology and Nematology, University of Florida, Lake Alfred, FL 33850, ³Centro de Investigaciones Biotecnológicas del Ecuador, Escuela Superior Politécnica del Litoral, Guayaquil, Ecuador.

Root-knot nematodes are globally considered one of the most devastating plant-parasitic nematodes and are responsible for significant economic losses on a multitude of crops including tomato. The use of resistant varieties is a key tactic in the arsenal of management tools to control root-knot nematodes. In tomato, a single dominant gene referred to as the *Mi* gene has been widely used in plant breeding efforts and varietal development, which confers resistance to a number of the most economically importance species of root-knot nematode found in Florida, including *Meloidogyne incognita*, *arenaria*, and *jananica*. *Mi*-virulent, resistance breaking isolates of these species, capable of reproduction and causing plant damage, have been detected in many areas of the world following the repeated use of these cultivars in field production. All too frequently, the discovery is also made that field populations of many different species of root-knot nematode are present in production fields, and those species that overcome the *Mi* resistance gene can proliferate and cause damage. *M. haplanaria* is an example of a root-knot nematode species recently reported in Florida affecting tomato crops that carry the *Mi* gene; this species was originally reported affecting peanut crops in Texas and Arkansas. Little is known about this nematode and its potential effect on tomato cultivars, including those conferred with the *Mi* gene. The main goal of the studies reported herein was to quantitatively describe the relationship between eight initial population densities of *M. haplanaria* on the resistant tomato cultivar “Sanibel” and the non-resistant cultivar “Rutgers”. Results from greenhouse and growth chamber studies will also be discussed.

THE GENOME OF THE YELLOW POTATO CYST NEMATODE, *GLOBODERA ROSTOCHIENSIS*, REVEALS INSIGHTS INTO THE BASES OF PARASITISM AND VIRULENCE. **Eves-van den Akker, S.¹, D.R. Laetsch², P. Thorpe³, C.J. Lilley⁴, E.G.J. Danchin⁵, M. DaRocha⁵, C. Rancurel⁵, N.E. Holroyd⁶, J.A. Cotton⁶, A. Szitenberg⁷, E. Grenier⁸, J. Montarry⁸, B. Mimee⁹, M. Duceppe⁹, I. Boyes¹⁰, J.M.C. Marvin⁴, L.M. Jones⁴, H.B. Yusup⁴, J. Lafond-Lapalme⁹, M. Esquibet⁸, M. Sabeh⁹, M. Rott¹⁰, H. Overmars¹¹, A. Finkers-Tomczak¹¹, G. Smant¹¹, G. Koutsovoulos², V. Blok³, S. Mantelin³, P.J.A. Cock¹², W. Phillips¹³, B. Henrissat^{14,15}, P.E. Urwin⁴, M. Blaxter², and J.T. Jones^{3,16}.** ¹Division of Plant Sciences, College of Life Sciences, University of Dundee, Dundee, DD1 5EH, UK, ²Institute of Evolutionary Biology, University of Edinburgh, EH9 3FL, UK, ³Cell and Molecular Sciences Group, Dundee Effector Consortium, James Hutton Institute, Dundee, DD2 5DA, UK, ⁴Centre for Plant Sciences, University of Leeds, Leeds, LS2 9JT, UK, ⁵INRA, Univ. Nice Sophia Antipolis, CNRS, UMR 1355-7254 Institut Sophia Agrobiotech, 06900 Sophia Antipolis, France, ⁶Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Cambridge, CB10 1SA, UK, ⁷School of Biological, Biomedical and Environmental Sciences, University of Hull, Hull, HU6 7RX, UK, ⁸INRA, UMR1349 IGEPP (Institute for Genetics, Environment and Plant Protection), F-35653 Le Rheu, France, ⁹Agriculture and Agri-food Canada, Horticulture Research and Development Centre, 430 Bboul. Gouin, St-Jean-sur-Richelieu, Quebec, J3B 3E6, Canada, ¹⁰Sidney Laboratory, Canadian Food Inspection Agency (CFIA), 8801 East Saanich Rd, Sidney, BC V8L 1H3, Canada, ¹¹Laboratory of Nematology, Department of Plant Sciences, Wageningen University, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands, ¹²Information and Computational Sciences Group, James Hutton Institute, Dundee, UK, ¹³USDA-ARS Horticultural Crops Research Laboratory, Corvallis, Oregon, USA, ¹⁴CNRS UMR 7257, INRA, USC 1408, Aix-Marseille University, AFMB, 13288 Marseille, France, ¹⁵Department of Biological Sciences, King Abdulaziz University, Jeddah, Saudi Arabia, ¹⁶School of Biology, University of St Andrews, North Haugh, St Andrews, KY16 9TZ, UK.

The yellow potato cyst nematode *Globodera rostochiensis* is a devastating plant pathogen of global economic importance, classified into pathotypes of different plant resistance-breaking phenotypes. *G. rostochiensis* secretes effectors, some of which were acquired by horizontal gene transfer (HGT), from pharyngeal glands into the host to manipulate host processes and promote parasitism. We generated a high-quality genome assembly for *G. rostochiensis* pathotype Ro1 and identified putative effectors and HGT events, mapped gene expression through the life cycle focusing on key parasitic transitions, and sequenced the genomes of eight populations including three additional pathotypes. HGT contributed 3.5% of the predicted genes, ~8.5% of which are deployed as effectors. Over one third of all effectors were clustered in 21 “effector islands”. We identified a motif (DOG box) present upstream of representatives of 26 of 28 dorsal-gland effector families, and predicted a superset of putative effectors associated with this motif. We validated gland cell expression for two novel genes by *in situ* hybridisation, and catalogued DOG effectors from available cyst nematode genomes. Comparison of effector diversity between pathotypes highlights those which correlate with plant resistance-breaking. These resources rapidly establish *G. rostochiensis* as a model to study pathogenicity and virulence in plant-parasitic nematodes.

THE TRANSCRIPTOME OF *NACOBBUS ABERRANS* REVEALS INSIGHTS INTO THE EVOLUTION OF SEDENTARY ENDOPARASITISM IN PLANT-PARASITIC NEMATODES. **Eves-van den Akker, S.¹, C.J. Lilley², E.G.J. Danchin³, C. Rancurel³, P.J.A. Cock⁴, P.E. Urwin² and J.T. Jones^{5,6}.** ¹Division of Plant Sciences, College of Life Sciences, University of Dundee, Dundee, DD1 5EH, UK, ²Centre for Plant Sciences, University of Leeds, Leeds, LS2 9JT, UK, ³INRA, Univ. Nice Sophia Antipolis, CNRS, UMR 1355-7254 Institut Sophia Agrobiotech, 06900 Sophia Antipolis, France, ⁴Information and Computational Sciences Group, James Hutton Institute, Dundee, UK, ⁵Cell and Molecular Sciences Group, Dundee Effector Consortium, James Hutton Institute, Dundee, DD2 5DA, UK, ⁶School of Biology, University of St Andrews.

Within the phylum Nematoda, sedentary plant-parasitism is hypothesised to have arisen independently on at least four occasions. The most economically damaging plant-parasitic nematode species, and consequently the most widely studied, are those that feed as they migrate destructively through host roots causing necrotic lesions (migratory endoparasites) and those that modify host root tissue to create a nutrient sink from which they feed (sedentary endoparasites). The false root-knot nematode *Nacobbus aberrans* is rare in that it has both migratory endoparasitic and sedentary endoparasitic stages within its life cycle. It is widely accepted that the cyst and root-knot nematodes evolved sedentary endo-parasitism independently, as evidenced by essentially no overlap in effector repertoires, and distinctly different complements of cell wall degrading enzymes acquired via horizontal gene transfer. While the sedentary stage of *Nacobbus* appears to phenotypically have characteristics of both the root-knot and the cyst nematodes, genetic data we present supports this “intermediate” position. We present the first large scale genetic resource of any false-root knot nematode species. We used RNAseq to describe relative abundance changes in all expressed genes across the life cycle to provide interesting insights into the biology of this nematode as it transitions between modes of parasitism. A multi-gene phylogenetic analysis of *N. aberrans* with respect to plant-parasitic nematodes of all groups confirms its proximity to both cyst and root-knot nematodes. We present a transcriptome-wide analysis of both lateral gene transfer events and the effector complement. Comparing parasitism genes of typical root-knot and cyst nematodes to those of *N. aberrans* has revealed interesting similarities: genes that were believed to be either cyst nematode- or root-knot nematode-specific have both been identified in *N. aberrans*. Our results may provide insights into the characteristics of a common ancestor and the evolution of sedentary endoparasitism of plants by nematodes.

FLUOPYRAM: A NEW NEMATICIDE FOR NEMATODE MANAGEMENT IN COTTON. Fasje, T.R.¹, K. Hurd¹, and M. Emerson¹. ¹University of Arkansas, Lonoke Research and Extension Center, Lonoke, AR.

The loss of aldicarb has greatly impacted nematode management in cotton. A potential replacement for aldicarb is fluopyram, a succinate dehydrogenase inhibitor (SDHI) fungicide, which has been shown to affect plant-parasitic nematodes. A liquid formulation of fluopyram + imidacloprid (Velum Total[®], Bayer CropScience) was registered in 2015 for use against plant-parasitic nematodes and early-season insects in cotton. Since 2013, several field and greenhouse experiments have been conducted using fluopyram for the management of root-knot nematode (*Meloidogyne incognita*) and reniform nematode (*Rotylenchulus reniformis*). Our results indicate that fluopyram affects nematode motility and effective in protecting the cotton root system in greenhouse trials. The field performance of this formulation was often more effective in suppressing root-knot and reniform nematode reproduction on cotton than existing commercial seed treatment nematicides. Results from these trials and nematode response to different rates on cotton will be presented. The utility of this formulation and fluopyram-treated cotton seed will be discussed for use in the Mid-South cotton production system.

INTEGRAL FAUNAL ANALYSIS BASED ON NEMATODE ASSEMBLAGES. Ferris, H. Department of Entomology and Nematology, University of California, Davis, CA 95616.

The evolving field of integral faunal analysis (IFA), using nematodes as bioindicators of soil condition and soil health, has three components. Each has a basis in observation, experimentation, and ecological principle, yet each component involves some heroic assumptions and sweeping generalizations. These represent rich areas for research, verification and validation. The components of IFA are (i) proportional faunal analysis, the partitioning of the nematode assemblage into functional guilds; (ii) calculation of the functional magnitude of each guild; and (iii) determination of the species diversity within each guild as a measure of the spatial and temporal complementarity of functions. Researchable questions abound within this framework. In component (i), the level of taxonomic resolution at which nematodes are identified and assigned to functional guilds often is constrained by expertise and time commitment. The determination of life history strategies and feeding habits at the genus and species level will be important in improved resolution of bioindicator potential and model validation. The application of biochemical and molecular techniques in analysis of intestinal content may be very useful in determining feeding habits and therefore functional roles. In component (ii), biomass and metabolic footprint determinations often are based on dimensions of adult nematodes published in the original species descriptions and calculated as averages for the species in each group when taxonomic identification is at the genus or family level. Resolution will be improved by study of the differences in size and activity among species within a genus or family and by determination of the size and activity distribution among life stages of the current population. In component (iii), determining the ranges of morphological, physiological, and behavioral characteristics of species within functional guilds will provide greater understanding of the importance of species diversity with regards to spatial, seasonal and future climatic cycles as experienced in the three-dimensional matrix of soils. Important next steps in IFA development and application are to move beyond inferences based on the descriptive assessment of nematode assemblages in environments with edaphic differences or anthropogenic disturbances. We need to determine and validate the relationships between form and function; that requires experimental verification of the nature and magnitude of the ecosystem functions performed by the species assemblage in each nematode guild. Despite current data gaps and challenging uncertainties, the application of IFA provides a useful framework for inferring the condition of soils and for monitoring the effects of perturbation and management; it provides a road-map for future development.

HABITATS AND FOOD WEBS: SOIL WATER POTENTIAL DIFFERENTIATES THE RESPONSES OF TWO ALLOPATRIC ENTOMOPATHOGENIC NEMATODES TO AN HERBIVORE INDUCED PLANT VOLATILE. Filgueiras C., Camila, D.S. Willett, and L.W. Duncan. University of Florida, IFAS, Citrus Research and Education Center, Lake Alfred, FL 33850.

The entomopathogenic nematode *Steinernema diaprepesi* occurs more frequently in citrus orchards in the Florida central ridge ecoregion compared to those in the flatwoods. A closely related, undescribed steinernematid species is frequently recovered from flatwoods orchards, but rarely on the central ridge. The depth to groundwater is generally deeper on the central ridge than in the flatwoods and well drained vs poorly drained soils characterize the two regions, respectively. In the laboratory, *S. diaprepesi* orients toward and persists longer at lower water potential whereas *Steinernema* sp. exhibits the opposite behaviors. We speculated that soil moisture might modulate the responses to semiochemicals differently for each species and in ways that would best adapt each to its preferred habitat. Pregeijerene is a volatile terpenoid that is induced by root herbivory and can function indirectly in plant defense by attracting EPNs to the site of the injury. Attraction assays were done in washed, baked (260 °C, 3 h) sand adjusted to either 6% or 18% soil moisture. PVC T-tubes (3/4-inch diameter, ~3 inches long) were packed with the sand at either moisture. Filter paper containing the desired treatments (10 µl pentane vs 10 µl of 10ng/ul pregeijerene in pentane) was placed in sand-filled caps on opposing sides of the T-tubes and 500 infective juveniles of either *S. diaprepesi* or *Steinernema* sp. were released in the center. After 24 hours, EPNs in the caps were separated from sand by decanting and counted. As predicted, each species responded differently to pregeijerene in the drier

than in the wetter sand, and the behaviors of *S. diaprepesi* were opposite to those of *Steinernema sp.* Pregeijerene attracted *S. diaprepesi* (paired t-test $p = 0.02$) and repelled *Steinernema sp.* ($p = 0.04$) in wetter soil and it repelled *S. diaprepesi* ($p = 0.03$) and attracted *Steinernema sp.* ($p = 0.03$) in drier soil. It is unknown how the effects of moisture on orientation are adaptive for these species/habitat combinations. Moreover, the mechanism by which moisture interacts with plant volatiles to produce qualitatively different EPN behavior merits additional research.

DISTRIBUCIÓN DE *MELOIDOGYNE ENTEROLOBII* EN LAS ZONAS PRODUCTORAS DE GUAYABA (*PSIDIUM GUAJAVA*) EN COSTA RICA. Flores-Chaves, Lorena¹, M. Gómez¹, D.A. Humphreys-Pereira¹, L. Gómez-Alpizar², L. Salazar¹. ¹Laboratorio de Nematología-CIPROC, Universidad de Costa Rica, 2060 San Pedro, Costa Rica, ²Laboratorio de Biotecnología de Plantas-CIA, Universidad de Costa Rica, 2060 San Pedro, Costa Rica.

Meloidogyne enterolobii es un problema fitosanitario en plantaciones de guayaba; la presencia de este nematodo ocasiona pérdidas en producción de hasta un 50% y puede llegar a causar la muerte de plantas. En Costa Rica, las zonas productoras de guayaba se ubican en la provincia de Puntarenas, Alajuela, Cartago, Heredia y Limón, principalmente. La evidencia de sintomatología aérea en las plantas y daños en las raíces ocasionadas por *Meloidogyne sp.* provocó que a partir del 2010 se iniciara una investigación en plantaciones de guayaba criolla y cv. Tai-kuo-bar para determinar las especies asociadas a este cultivo. Las muestras de raíces agalladas se recolectaron de varias localidades: Paquera, Jicaral, Barrio San José, El Cacao, Poás, Tacares (Alajuela), Sarapiquí (Heredia), Guayabo, Mollejones, Pacuare, San Joaquín (Cartago), Guácimo, Guápiles y Pocora (Limón) entre otras. Para el diagnóstico, se extrajo ADN de hembras y se utilizaron las técnicas moleculares del PCR con imprimadores (C2F3/1108) y la secuenciación. En la mayoría de las localidades se identificó la presencia de *M. enterolobii*. Por la sintomatología observada en las raíces de corchosis se aislaron hongos y se realizaron cultivos mono-spóricos, PCR (imprimadores ef1/ef2) y secuenciación para determinar la posible asociación de hongos patogénicos con el nematodo. Los análisis mostraron que en la provincia de Alajuela la frecuencia relativa de *M. enterolobii* y *Fusarium oxysporum* fue de 88,9% y 33,3%, respectivamente. En la provincia de Cartago, *M. enterolobii* presentó una frecuencia relativa de 41,7% y *F. oxysporum* de 8,33%. En Puntarenas *M. enterolobii* y *F. oxysporum* se presentaron en el 40% de las muestras analizadas. En Limón la frecuencia de *M. enterolobii* fue de 100% y se determinó *F. proliferatum* en 25% de las muestras, no así *F. oxysporum*. En Heredia las muestras no evidenciaron presencia del nematodo. Estos resultados han permitido conocer la situación real que están enfrentando los productores de guayaba en Costa Rica ante la presencia de *M. enterolobii* y ha generado investigación enfocada en la búsqueda de materiales vegetales promisorios para el manejo de estenematodo.

DENSIDAD POBLACIONAL DEL NEMATODO QUISTE (*GLOBODERA* SPP.) EN EL CULTIVO DE PAPA (*SOLANUM TUBEROSUM* L.) DE LA REGIÓN PUNO-PERÚ. Flores Choque, Yeni F.¹, L.M. Israel¹, B.P. Rosario Ysabel¹, A.G. Marilia Isabel¹, M. C. Zheylya Danitza², G.A. Steward Irwin²; C.J. Shadam Elvis², and Z.T. Noely Carelim². Universidad Nacional del Altiplano Puno - Perú, ¹Facultad de Ciencias Agrarias, Escuela Profesional de Ingeniería Agronómica, ²Facultad de Ciencias Biológicas.

La producción del cultivo de papa en la región Puno del territorio peruano es propenso a plagas y enfermedades debido al mal manejo del cultivo por parte del agricultor. Uno de los principales problemas fitosanitarios que inciden sobre la producción de papa en los andes peruanos lo constituye el género *Globodera* spp, parásitos que causan daños significativos en el rendimiento y en la calidad de tubérculos. El objetivo del presente trabajo es evaluar la densidad poblacional del nematodo quiste (*Globodera* spp.) en el cultivo de papa de la región Puno – Perú. Se evaluaron 160 muestras de 7 provincias de la región Puno en la campaña agrícola 2015-2016. Las muestras de suelo colectadas fueron procesadas por el método de fluctuación centrífuga en solución. Los resultados demostraron que en altitudes que varían desde los 3824 a los 4376 msnm, la presencia del género *Globodera* spp. fue de 100% en las muestras, sin embargo las densidades poblacionales varían de acuerdo a la provincia evaluada; Carabaya, Yunguyo, Huancané, Puno, Chucuito, Azángaro y Sandía (295.64, 227.54, 208.79, 180.68, 180.62, 172.39 y 122.41 quistes/100 cm³ de suelo respectivamente); de ésta forma la producción y el bajo rendimiento no solo se debe a los factores climáticos de la zona sino también a las altas poblaciones de éste género de importancia agrícola que evitan el normal desarrollo de la planta del cultivo de papa.

EVALUACIÓN DE PRODUCTOS NEMATICIDAS, PLAGUICIDAS Y FUNGICIDAS EN EL RENDIMIENTO DE GRANO DE MAÍZ DE TEMPORAL. Flores-López, H.E., J. Ireta-Moreno¹, J.F. Pérez-Domínguez¹, N.Y. Zacamo-Velázquez¹, I. López-Caratachea¹, and A. Tejada². ¹ INIFAP-Campo Experimental Centro Altos de Jalisco, km 8 carretera Tepatitlán-Lagos de Moreno, Tepatitlán, Jalisco, México CP47600. ² The Seedcare Institute. Tlaquepaque, Jalisco, México.

Los hongos, nemátodos y/o plagas del follaje y suelo, son organismos dañinos que tienen influencia importante en el rendimiento del maíz, por lo que es necesario proveer de protección a este cultivo, particularmente en las primeras etapas del ciclo del cultivo cuando este es más vulnerable. El objetivo del presente estudio fue evaluar seis productos con propiedades fungicidas, nematicidas y plaguicidas, sobre el rendimiento de maíz de temporal. En 2015 se estableció un experimento en

maíz de temporal con diseño en bloques al azar y tres repeticiones, con fecha de siembra el 17 de junio. Los productos devaluación fueron tratamientos a la semilla con Fortenza Duo más Vibrance (FDV), Fortenza Duo más Force (FDF), Fortenza Duo (FD), Fortenza Duo más Avicta (FDA), Cruiser Maxx más Vibrance (CMV) e insecticida Furadan (FUN) aplicado al suelo. Durante el ciclo del cultivo se muestreo el suelo de cada tratamiento el 7 de julio, 11 de agosto, 1 de septiembre y 19 de octubre, para cuantificar los nemátodos presentes. Al final del ciclo se evaluó el rendimiento de grano. Ocurrió fuerte compactación, por lo que se midió la resistencia a la penetración en el suelo con penetrómetro. Los resultados muestran que no hubo diferencias significativas en el número de nemátodos con los tratamientos a la semilla en las fechas de muestreo. El rendimiento de grano mostró diferencias significativas al 5% de probabilidad, de acuerdo con el siguiente orden: con el más alto en FD, FDF, FDV, FDA, CMV e FUN, con valores de 11691, 10843, 10412, 9351, 9289, 8483 kg/ha, respectivamente. La tendencia del número de nemátodos en el primero y segundo muestreo (7 de julio y 11 de agosto), manifestaron tendencia a reducir el rendimiento de grano con el aumento de nematodos, mientras en el tercero y cuarto muestreo (1 de septiembre y 19 de octubre), no se encontró tendencia. En el tercer y cuarto muestreo de nemátodos se observó la ocurrencia de nemátodos muertos, con mayor proporción en el último muestreo. Se observó compactación del suelo, más alta en los tratamientos FDA, FDV y CMV, con valores de resistencia a la penetración de 2.28, 1.97 y 1.94 kg/cm², respectivamente.

INFLUENCIA DE LAS PRACTICAS DE MANEJO Y CANTIDAD DE NEMATODOS SOBRE EL RENDIMIENTO DE GRANO DE MAÍZ DE TEMPORAL. Flores-López, H.E.¹, J. Ireta-Moreno¹, J.F. Pérez-Domínguez¹, N.Y. Zacamo-Velázquez¹, I. López-Caratachea¹, and A. Tejada². ¹INIFAP-Campo Experimental Centro Altos de Jalisco, km 8 carretera Tepatitlán-Lagos de Moreno, Tepatitlán, Jalisco, México CP47600. ²The Seedcare Institute. Guadalajara, Jalisco, México.

El maíz es uno de los cultivos más importantes en México, el cual se reporta con significativos efectos de las plagas sobre el rendimiento. Los nemátodos se encuentran entre los microorganismos que afectan el rendimiento, pero en México poca ha sido la investigación desarrollada para evaluar esta interacción. El objetivo del presente estudio fue evaluar la interacción de las prácticas de manejo del cultivo de maíz y el número de nemátodos muestreados sobre el rendimiento de maíz. En 2015 se condujo un estudio en maíz de temporal con prácticas de manejo en maíz de temporal con diseño en bloques al azar y tres repeticiones, con fecha de siembra el 17 de junio. Se utilizaron 18 tratamientos que incluyeron labranza del suelo (convencional y conservación), cobertura del suelo en labranza de conservación con 0, 50 y 100% de residuos de cultivo, fertilización mineral y orgánica, surcos angosto y melonero, tratamiento a semilla para protección contra plagas y nemátodos. Durante el ciclo del cultivo se muestreo el suelo de cada tratamiento el 7 de julio, 11 de agosto, 1 de septiembre y 19 de octubre, para cuantificar los nemátodos presentes. Al final del ciclo se evaluó el rendimiento de grano. Los resultados mostraron que solo el primer muestreo de nemátodos fue significativo al 5% de probabilidad en el tratamiento a semilla y con surco angosto y melonero. El rendimiento grano resultó con significancia estadística en los siguientes casos: 1) las formas de labranza: a) con barbecho más dos pasos de rastra, b) con tres pasos de rastra y c) labranza de conservación; los rendimientos en estas labranzas fue de 10116, 9547 y 7949 kg/ha, respectivamente; 2) con el porcentaje de residuos de la cosecha en el tratamiento de labranza de conservación con 0, 50 y 100% de residuos, el rendimiento fue de 9357, 7515 y 7665 kg/ha, respectivamente; 3) en surco tradicional y tipo cama melonera, donde los rendimientos de grano fueron 8596 y 7908 kg/ha, respectivamente; 4) con fertilización mineral y fertilización orgánica el rendimiento fue de 9179 y 7555 kg/ha, respectivamente. El efecto del número de nemátodos mostró una tendencia a reducir el rendimiento solo con el primero muestreo en los tratamientos del tipo de labranza, porcentaje de residuos, surcado convencional y melonero, fertilización mineral y orgánica, tratamiento a semilla.

USING COMPOST SOIL AMENDMENTS IN AN INTEGRATED REPLANT MANAGEMENT PROGRAM FOR PERENNIAL FRUIT CROPS: EFFECTS ON PLANT-PARASITIC NEMATODES. Forge, T.¹, T. Watson², D. Neilsen¹, G. Neilsen¹, L. Nelson², P. Munroe², and P. Randall¹. ¹Agriculture and Agri-Food Canada, 4200 Hwy 97, Summerland, BC V0H 1Z0. ²Biology Department, University of British Columbia-Okanagan, Kelowna, BC V1V 1V7.

Increasing restrictions on the use of broad spectrum fumigants have highlighted the need to develop more sustainable methods of suppressing plant-parasitic nematodes and associated replant disease complexes prior to replanting perennial fruit crops. Two field experiments were established in the Okanagan Valley of British Columbia in 2014 and 2015 to study the interactive influences of prior soil organic matter management, at-plant compost amendments, post-plant applications of organic mulches, and alternative micro-irrigation practices on population densities of plant-parasitic nematodes, selected soil health indicators and establishment of cherry trees replanted into old orchard sites. Each experiment included fumigated plots as positive controls. In both experiments, population densities of *Pratylenchus penetrans*, which is widely associated with poor replant establishment of temperate fruit trees, were suppressed in compost-amended soil relative to non-amended soil. First-year tree growth in compost-amended plots was intermediate between fumigated and non-amended plots. At the end of the second growing season of the 2014 experiment, tree growth in compost and compost + mulch plots was not different from fumigated plots. *P. penetrans* population densities increased rapidly in fumigated plots in the 2014 experiment, exceeding those in compost-amended plots by the end of the

second growing season. Similarly, in the 2015 experiment, population densities of *Paratrichodorus* sp. were greater at the end of the first growing season in fumigated plots than in non-fumigated plots. Additional analyses indicate that compost amendments can have favourable effects on soil nutrient availability, water relations and the composition of the rhizosphere bacterial community, increasing the prevalence of total bacteria, 2,4-diacetylphloroglucinol-producing bacteria, and pyrrolnitrin-producing bacteria, which have been associated with rhizosphere suppression of fungal root pathogens as well as parasitic nematodes. Compost amendments did not significantly enhance free-living soil nematode populations in the first growing season after application and planting at either site. The importance of parasitic nematode suppression for enhanced replant establishment of fruit trees will be discussed relative to the multiple other benefits of soil organic matter enhancement.

INTEGRATED MANAGEMENT OF *NACOBBUS ABERRANS* IN THE ANDEAN REGION OF SOUTH AMERICA. Ponce, J.F. PROINPA, Casilla 4285, Cochabamba, Bolivia.

Undoubtedly, among the main limitations of potato cultivation in the Andean region of Latin America and present in geographical areas with specific agro-ecological conditions, is the “potato rosary nematode”, *Nacobbus aberrans*. In the temperate Andean region of Latin America, *Nacobbus aberrans sensu lato*, also known as the “false root-nematode”, has more than 80 species of cultivated and non-cultivated plants as hosts. In addition to the cultivated potato (*Solanum tuberosum* ssp. *andigena*), the false root-knot nematode causes damage to other traditional Andean tubers such as oca (*Oxalis tuberosa*), mashua or isaño (*Tropaeolum tuberosum*), olluco or papalisa (*Ullucus tuberosus*) and grains like quinoa (*Chenopodium quinoa*). Extrapolation of all collected and systematized information data on distribution (incidence) and yield losses (severity) caused by *N. aberrans s. l.* in potato production in Bolivia, cultivated areas and selling price of tubers were used to estimate that economic losses in the gross value of potato production was of US \$ 53 000,000 in areas which were mostly located between 3000-4000 meters above sea level. Integrated management of *N. aberrans s. l.* was implemented by crop rotations with non-host plants and / or trap plants. Studies of interactions between these nematodes and Andean crops allowed to identify lines or cultivars of those crops, which behaved as resistant, traps or antagonistic. In addition, several alternatives have been evaluated (i.e., incorporation of manure, green matter, elimination of infected roots, extraction of volunteer plants, resistant varieties, and treatment of infected tubers), and expected to be adopted by farmers. As indicated, it is necessary to continue the search for alternatives such as the use of plant growth promoter micro-organisms and / or suppression of plant pathogens (bacteria and mycorrhizal fungi), and / or genetic engineering (generation of resistant varieties); which, if practicable, their incorporation as new integrated management components will need of serious studies on risk assessments for the environment, biodiversity and human health. Also, even if their distribution is restricted to the Americas, there is a demand for quarantine efforts to prevent the entry of *Nacobbus aberrans s. l.* into countries of the European Community and others. Isolated local efforts must be approached from a regional angle. In this sense, for the development and implementation of a regional strategy to *N. aberrans s. l.*, PROINPA in Bolivia has been conducting informal proposals to promote the establishment of a regional project to coordinate and optimize the technological advances made at the level of each country.

GLOBODERA SPP: IDENTIFICATION, DISTRIBUTION AND HOST IN THE ANDEAN REGIONS. Ponce, J.F. PROINPA, Casilla 4285, Cochabamba, Bolivia.

Potato cultivation is carried out under a wide range of agro-ecologies that outperform other crops of global importance in Latin American countries. Among the most important constraints in the Andean region of Latin America are the nematode species *Globodera rostochiensis* and *G. pallida* (potato cyst nematodes), due to their distribution, the existence of pathogenic races, dissemination, difficult diagnosis, and the economic losses they cause. Among the most comprehensive studies in Latin America in relation to *Globodera* spp. are those made in Bolivia and Peru, which by extrapolation of cultivated areas, incidence and severity of damage by nematodes and selling price of tubers, were used to estimate the economic losses these cause in the gross value of potato production. For several years, soil samplings were conducted with Andean native and improved potato producers in ten major departments of Peru. All departments showed the presence of potato cyst nematodes and 64.9% of the 3299 samples tested were positive, and the effect of their incidence and severity on potential losses in crop yield of improved and native potatoes in the Andean region of Peru was analyzed. The dominant species was *G. pallida* relative to *G. rostochiensis* and both species were more frequent between 3,500 and 4,000 meters above sea level. In most cases, losses occurring in Peru refer to those caused by *G. pallida* and were estimated at US \$128,000,000. Studies conducted in similar way estimated a total potato yield loss of US \$13,000,000 in Bolivia. To implement an integrated management of *Globodera* spp., several alternatives, such as incorporating manure, green matter, extraction of volunteer plants have been evaluated. The availability of lines or cultivars of Andean crops that present a different behavior in relation to nematodes show that these lines can be incorporated into rotation systems. In relation to the use of these Andean crops, new concepts on host-parasite relationships are presented between *Globodera* spp., with Andean crops in traditional farming systems (oca, isaño or mashua, olluco or papalisa, quinoa and lupinus or tarwi, also introduced crops such as barley, wheat, bean).

POCHONIA CHLAMYDOSPORIA VS NACOBBUS ABERRANS: EXPERIENCES IN THE CONTROL OF THE FALSE ROOT-KNOT NEMATODE IN MEXICO. Franco-Navarro, F., R. Velasco-Azorsa, and I. Cid del Prado-Vera. Plant Pathology Program, Colegio de Postgraduados-Campus Montecillo, Mexico State, Mexico 56230.

One of the most important plant-parasitic nematodes in Mexican agriculture is the false root-knot nematode, *Nacobbus aberrans*, which causes severe damage to crops such as tomato, chili and beans. Chemical control of this nematode is more or less effective but costly, and has associated environmental and human health concerns. As a consequence, biological control strategies could become more attractive to Mexican growers in production systems infested with false root-knot nematode. In this context, some fungi are recognized as promising biological control agents for plant-parasitic nematodes. One of them, *Pochonia chlamydosporia*, a facultative parasite present in many soils, colonizes the rhizosphere of several crops and proliferates in egg masses deposited by those nematodes infecting the roots of their hosts; moreover, it has been successfully applied to soil as a chlamydospore suspension to reduce root-knot populations and their damage to susceptible plants. Mexican native isolates of *P. c. var. chlamydosporia* from five states (Mexico State, Puebla, Tlaxcala, Veracruz and Zacatecas) have been tested for the biological control of *N. aberrans*; we present a synoptic perspective of the potential of *P. c. var. chlamydosporia* as a biological control agent of the false root-knot nematode. In Mexico, we have: 1) studied the cultural and biological characteristics of isolates, and their parasitic potential; 2) standardized methods for mass production of the fungus; 3) determined the colonizing ability of roots by Mexican isolates and their crop range; 4) measured parasitism of *N. aberrans* eggs from different populations; 5) applied fungus to plant seedbeds; 6) tested the biological efficiency against the false root-knot nematode under greenhouse conditions on tomato, chili and beans; 7) tested biological management of *N. aberrans* with the fungus under field conditions (chili); and 8) incorporated the fungus into an Integrated Control scheme for *N. aberrans* by combination with nematicide application, and the incorporation of cabbage residues and composted manures. In summary, from our experiences and results, successful strategies have emerged for the Integrated Control of *N. aberrans* using *P. chlamydosporia* in combination with other eco-friendly approaches.

INVESTIGACIÓN CIENTÍFICA APOYADA POR CNPQ EN NEMATOLOGÍA POR MEDIO DE CONVOCATORIA UNIVERSAL. Freitas, V. Moreira de. Consejo Nacional de Desarrollo Científico y Tecnológico (CNPq), Brasília, Distrito Federal.

El CNPq es una Fundación Pública Brasileña que promueve la ciencia en diferentes áreas del conocimiento, en distintas regiones geográficas, aprobando recursos financieros en partidas de capital, costeo y becas. El programa básico en Agronomía (AG), vinculado a la Dirección de Ciencias Agrícolas (DABS), tiene un comité asesor (CA), formado por investigadores de reconocido prestigio pertenecientes a la comunidad científica de Agronomía. Cuando el CNPq publica una convocatoria pública, las propuestas son analizadas cuanto al mérito científico por el CA. Sin embargo, la decisión final sobre la concesión de fondos se hace por la DABS. Este resumen tiene como objetivo divulgar los datos de las cuatro últimas selecciones de la Convocatoria Universal (CU). En la CU de 2012, 17507 propuestas se presentaron al CNPq, siendo que 1178 fueran presentadas al programa AG, 275 fueran aprobadas y solamente diez eran relacionados a la nematología (seis del Medio-Oeste, una del Noreste y tres del Sureste brasileño). En la CU de 2013 fueron sometidas al CNPq 17813 propuestas, 1134 en el programa AG, solamente 286 fueran aprobadas, siendo siete relacionadas a nematología (tres del Sureste, dos del Medio-Oeste y dos del Sur). En la CU de 2014 18286 propuestas se presentaron al CNPq, siendo 1044 presentadas al programa AG, solamente 278 fueran aprobadas, siendo siete propuestas en nematología (dos del Noreste, tres del Medio-Oeste, dos del Sureste). En 2015 la CU fue cancelada y reabierta en 2016 debido a la crisis política-económica brasileña. La CU de 2016 aún esta en curso, con resultados programados para julio de 2016. Con el análisis de los datos, se constató un crecimiento continuo en el número de propuestas presentadas al CNPq y decreciente en las sometidas al programa AG, a lo largo de los años. Pero, las propuestas aprobadas en agronomía y también en las aprobadas en nematología, específicamente, mantienen crecimiento constante, con ligeras variaciones. No hubo propuestas aprobadas en el Norte de Brasil, a pesar de la ley brasileña 11.540/2007 destinar cuota mínima de 30% de los recursos para investigadores del Norte, Noreste y Medio-Oeste Brasileño. Estos resultados ayudan a comprender el desarrollo en Nematología en Brasil.

POPULATION DYNAMICS OF *BELONOLAIMUS LONGICAUDATUS* IN CENTRAL NORTH CAROLINA. Galle, G., C.H. Opperman, and J.P. Kerns. Dept. of Plant Pathology, North Carolina State University, Raleigh, NC 27695.

The turfgrass industry in North Carolina is valued at an estimated \$4 billion dollars, and the grassy foundation it is built on is constantly under pathogen pressure. Sting nematode (*Belonolaimus longicaudatus*) is one of many damaging turf pathogens, but is problematic due to their high damage potential at very low numbers. Very little is known about *B. longicaudatus* in North Carolina, nor how they handle the cold of the winter season. A better understanding of nematode population dynamics at cool climates will lead to more effective control. This study's objectives are to identify nematode population numbers over the year, and to understand the vertical distribution of the nematodes within a putting green soil column. Four golf courses in Central North Carolina were sampled monthly at three different depths. *B. longicaudatus* populations were consistent among the golf course, as numbers ranged from 0-40 nematodes per 500cc soil during the winter and reached as high as 225 in the summer. Vertical distribution of sting nematode varied by golf course, with three out of four

showing a majority of nematodes residing in the 0-10 cm depth. However, one golf course that uses unique cultural practices kept most of the nematode population between 10-20 cm, which is the extent of turfgrass rooting. This result suggests that cultural control can have a large impact on nematode location, and the potential for damage resulting from feeding.

NEMATODOS FITOPARASITOS ASOCIADOS AL CULTIVO DE CAFÉ (*COFFEA ARABICA* L.) EN EL VALLE DE TAMBOPATA. **Garambel-Acurio, S.¹, L.M. Israel¹, B.P. Rosario¹, Z.T. Noely¹, F.CH. Yeni¹, A.G. Marilia¹, M.C. Zheyla¹, C. J. Shadam¹.** Universidad Nacional del Altiplano, Perú Unap, Peru.

Los nematodos proveen servicios claves al suelo como la supresión de patógenos y mantienen la fertilidad del suelo, por otra parte los nematodos Fitoparásitos causan grandes pérdidas al cultivo de café, al impedir el paso de nutrientes y el normal desarrollo de la planta; ocasionando pérdidas en la producción del cultivo y alentando la proliferación de otras plagas y enfermedades. El objetivo del presente trabajo fue identificar los diferentes géneros de nematodos fitoparásitos asociados al cultivo de café en el Valle de Tambopata Puno- Perú. Se evaluaron 129 muestras de 3 provincias de la Región Puno. Estas fueron procesadas por el método de fluctuación centrífuga en solución de sacarosa y finalmente ser contados en un estereoscopio. Se identificaron los géneros de nematodos Fitoparásitos como: *Meloidogyne* spp., *Helicotylenchus* spp., *Pratylenchus* spp., *Xiphinema* spp., *Mesocriconema* spp., *Dorylaimus* spp., *Tylenchus* spp., *Hemicycliophora* spp., *Mononchus* spp., y nematodos de vida Libre (99.9, 97, 16.7, 22.5, 55, 4.9, 1.7, 3.8, 7.7 y 98% respectivamente). Se observó que los géneros *Meloidogyne* spp. y *Helicotylenchus* spp., presentaron un alto número de nematodos en las diferentes muestras analizadas.

EFFECTOR VARIATION AMONG POPULATIONS OF THE SOYBEAN CYST NEMATODE THAT DIFFER IN VIRULENCE. **Gardner, M.¹, E. Davis², T. Baum³, and M.G. Mitchum¹.** ¹Division of Plant Sciences and Bond Life Sciences Center, University of Missouri, Columbia, MO 65211; ²Department of Plant Pathology, North Carolina State University, Raleigh, NC, 27695; ³Department of Plant Pathology and Microbiology, Iowa State University, Ames, IA 50011.

Resistant soybean cultivars are a primary management strategy used to reduce crop damage from the soybean cyst nematode (SCN) *Heterodera glycines*. However, repeated use of resistant cultivars selects for populations of SCN that are able to successfully colonize and reproduce on the roots of these plants, reducing the efficacy of resistance. A repertoire of effectors secreted by SCN are essential for successful parasitism, but little is known about the level of sequence complexity among effectors within and across populations of the nematode that might promote virulence on a SCN-resistant soybean cultivar. In the absence of an available annotated SCN genome we performed *de novo* transcriptome assembly of the early parasitic stage of juveniles from three SCN populations virulent on Peking, PI88788, or PI437654-derived resistant soybean as well as one SCN population avirulent on all three of these soybean lines. By mining these transcriptomes, we identified multiple sequence variants of known effectors, determined the level of conservation of these effectors at the population level, and grouped them into effector families based on sequence similarity. Some of these families show an overall increase in variation within virulent populations while other effector family sequences are extremely highly conserved. By better understanding nematode effector variation, we can distinguish between effectors that co-evolve with the host genotype and those conserved by the pathogen to maintain a core function in parasitism.

THE COMPOSITION OF FIELD POPULATIONS OF THE POTATO CYST NEMATODE *GLOBODERA PALLIDA* IN THE UK. **Gartner, U. and V. Blok.** The James Hutton Institute, Invergowrie, Dundee DD2 5DA, UK.

Globally, potatoes are the most important non-cereal food crop, and in the UK more than 6 million tons of tubers are produced per year. Controlling pests and diseases of potato is challenging and requires on-going monitoring of populations to ensure appropriate control strategies are employed. The potato cyst nematodes (PCN) *Globodera pallida* and *G. rostochiensis*, which originate from South America, cause significant losses for the potato industry. Genetic studies have shown that there have been one introduction of *G. rostochiensis* and three of *G. pallida* into the UK, however the geographic distribution of the latter in the UK has not been determined. We are using molecular markers to determine their distribution and we are reassessing the virulence of UK field populations towards the main sources of resistance used in UK potato breeding programs. This approach may reveal new or hybrid populations and will provide potato breeders with appropriate populations for use in resistance assays. The James Hutton Institute PCN collection contains *G. pallida* populations with Pa1, Pa2 and Pa3 phenotypes, which have been collected from different sites in the UK over 50 years. We have used a Terminal Restriction Fragment Length Polymorphism (T-RFLP) assay to examine the composition of these populations. This assay is based on SNP variants in mitochondrial DNA of *G. pallida*. Interestingly, we have noted a change in the composition in populations collected from the same field, with a more complex composition in the recently collected population than in the historic samples. Our results indicate that current UK field populations of *G. pallida* are typically composed of more than one introduction from South America. This complexity in field samples raises the possibility of hybridisation between the different genotypes of *G. pallida* that coexist in the same field and the potential for the generation of novel genotypes with new phenotypes. The *G. pallida* Pa1, Pa2 and Pa3 pathotypes were defined by their virulence towards different resistances. This requires a laborious bioassay and few laboratories have access to the differential clones used in the test. We are

determining whether a molecular assay provides a proxy for this virulence assay. To facilitate this, single cyst lines were produced from various recently infested potato fields and from our JHI PCN collection. These have been characterised with the T-RFLP assay and are currently being phenotyped. This reassessment of current UK PCN field populations will ensure that resistance used in potato breeding programs is suitable and will provide broad-spectrum and durable resistance for *G. pallida*. This is particularly relevant for the development of new potato cultivars with high levels of durable resistance to PCN.

XENORHABDUS BACTERIAL SYMBIONTS FROM STEINERNEMA CLADE I NEMATODES KILL CLADE III STEINERNEMA FELTIAE NEMATODES USING DISTINCT MECHANISMS. Ginete, D.¹, K.E. Murfin^{1,2}, and H. Goodrich-Blair¹. ¹Department of Bacteriology, University of Wisconsin-Madison, Madison, WI, 53706, ²Department of Internal Medicine, Yale University, New Haven, CT, 06519.

Steinernema spp. entomopathogenic nematodes associate with specific species of *Xenorhabdus* bacteria. Conversely, certain *Xenorhabdus* spp. can associate with multiple *Steinernema* nematode spp. For example, *X. bovienii* bacteria associate with eight different *Steinernema* nematode species from distinct phylogenetic clades I and III. Previous work established that *X. bovienii* isolated from *S. intermedium*, a member of clade I, can kill *S. feltiae* nematodes, a member of clade III (Murfin *et al.*, 2015 and Murfin, Ginete, *et al.*, in preparation). To test if this killing phenotype was conserved among clade I *X. bovienii* symbionts, we tested if three additional bacterial isolates from clade I nematodes are lethal toward *S. feltiae* nematodes. We found that all three tested strains of *X. bovienii* bacteria isolated from three isolates of the clade I nematode *S. affine* can also kill *S. feltiae* nematodes. To assess how clade I bacterial symbionts kill *S. feltiae* nematodes, we performed survival assays on adult *S. feltiae* nematodes transferred onto lawns of clade I bacterial symbionts. We found that the *X. bovienii* strains from *S. affine* varied in their infection characteristics and the mortality they caused in *S. feltiae* nematodes, indicating that they kill through distinct mechanisms. Ongoing work suggests that one mechanism is an intestinal infection process mediated by a Shiga toxin homolog. Finally, we did a converse cross and tested if clade III bacterial symbionts can also inhibit clade I *S. affine* nematodes. We found that clade III bacterial symbionts support growth of *S. affine* nematodes, indicating that symbiont-mediated interclade nematode killing may be restricted to clade I bacterial symbionts. Overall our data support the notion that strain differences in *X. bovienii* bacteria can have significant effects on their *Steinernema* nematode hosts and their competitors, and further highlight the impact of bacterial strain variation in host-symbiont associations and their ecology.

A SURVEY OF APHELENCHOIDES BESSEYI ON RICE IN LOUISIANA. Godoy, F.M.C.¹, C. Overstreet¹, E.C. McGawley¹, D.M. Xavier¹, and M.T. Kularathna¹. ¹LSU Agricultural Center, Department of Plant Pathology and Crop Physiology, 302 Life Sciences Building, Baton Rouge, LA 70803.

Aphelenchoides besseyi, the causal agent of white-tip disease of rice is one of the plant pathogenic nematodes that causes losses in rice production. This nematode has historically been considered a minor pest in the United States but recently has been found in a number of quarantine samples for overseas shipment in both Arkansas and Louisiana. The objective of this study was determine the current distribution of *A. besseyi* in Louisiana. A survey was conducted using seed samples obtained from the State Seed Testing Laboratory of the Louisiana Department of Agriculture and Forestry. These seeds represent rice cultivars that were grown in Louisiana or imported from out-of-state for sale to farmers for the years 2015 and 2016. The samples were taken to the nematology lab at LSU and 25 gram sub-sample of each sample was analyzed. The sub-samples were blended in water, placed on a Baermann funnel and counted using a microscope after 24 hours. In total, 189 samples were evaluated, representing 25 different cultivars. Among these samples, 21% were from 4 hybrid cultivars imported from out-of-state. *Aphelenchoides besseyi* was found in 17 samples belonging to 6 cultivars. Fourteen of the infested samples were from the hybrid cultivars of rice. Approximately 83% of the samples from the rice hybrid cultivar XL 729 were infested with *A. besseyi*. Populations of the nematode ranged from 2 to 288 per 25g of infected rice seed. Most of the infested seed were from long grain types, but 3 of the infested samples were medium grain types. This study would indicate that most of the rice cultivars produced in Louisiana and released to farmers are relatively free of this pest. Future work will be conducted in order to evaluate more cultivars from Louisiana and test their resistance to white-tip nematode.

SURVEY OF PLANT-PARASITIC NEMATODES IN PULSE CROP FIELDS OF THE CANADIAN PRAIRIES. Gouvea Pereira, F. and M. Tenuta. Department of Soil Science, University of Manitoba, Winnipeg, MB, Canada R3T 2N2.

The quarantine pest nematode, *Ditylenchus dipsaci*, can hamper securing export markets for some crops. In Canada, we recently reported that the previous identification of *D. dipsaci* in yellow pea export shipments was likely the non-quarantine species, *D. weischeri*, a parasite of creeping thistle and not crops. To further understand the distribution and host preference of *D. weischeri* and other plant nematodes in the Canadian Prairies, a survey is being conducted on commercial pulse crop (lentil, pea, chickpea and faba bean) fields in Alberta, Saskatchewan, and Manitoba. A total of 187 samples of pea, chickpeas, lentils and thistle plants from 45 fields have been examined. Samples of pulse and creeping thistle plants (leaves, stems and roots) and soil samples were collected from each field. Nematodes from plant materials were recovered using the Baermann pan method and nematodes from soil using sugar-flotation followed by centrifugation. Recovered nematodes were identified to genus by morphological features. Molecular analysis by species-specific PCR, PCR-RFLP and sequencing of the ITS

(ITS1 + 5.8S + ITS2) of the rRNA gene were also used to identify recovered nematodes to species. Five genera of plant-parasitic nematodes have been recovered from plant materials so far: *Aphelenchoides*, *Aphelenchus*, *Ditylenchus*, *Paratylenchus*, and *Subanguina*. The genus *Aphelenchoides* had the highest frequency, and was found in 17% of plant samples, *Ditylenchus* at 14%, and *Paratylenchus* the lowest at 0.5%. Recovered nematode densities ranged between 1 and 10,000/5g plant material. Molecular analysis results to date indicate recovery of *D. dipsaci* (14/5g leaves) from one yellow pea field and *D. weischeri* (10,000 /5g thistle flowers) in three Manitoba pea fields. *D. dipsaci* has recently been reported in two garlic fields in southern Manitoba and *D. weischeri* is not considered an agricultural pest. Nematodes from plant and soil collected in 2015 continue to be analyzed in addition to new sampling conducted in 2016 to address the gap in understanding the distribution of plant-parasitic nematodes in pulse crops of the Canadian Prairies.

SHORT-TERM EFFECTS OF COVER CROPPING ON ROOT-LESION NEMATODE, STUNT NEMATODE AND SOIL ECOLOGY IN MICHIGAN CARROT PRODUCTION. **Grabau, Z.J.^{1,2}, Z.T.Z. Maung^{1, 3}, D.C. Noyes¹, D.G. Baas⁴, B.P. Werling⁵, D.C. Brainard¹, and H. Melakeberhan¹**. Michigan State University Department of Horticulture, East Lansing, MI, USA¹; University of Florida Department of Entomology & Nematology, Gainesville, FL, USA²; Department of Nematology, Kearney Agriculture and Research Extension Center, University of California - Riverside, Parlier CA, USA³; Michigan State University Extension-St. Joseph County, Centreville, MI, USA⁴; Michigan State University Extension-Oceana County, Hart, MI, USA⁴.

Cover crops are often used for reducing soil erosion, retaining soil nutrients, and building organic matter. Some cover crop cultivars are also marketed for managing plant-parasitic nematodes. This study assessed the impact of common Michigan cover crops on plant-parasitic nematodes and soil ecology based on the nematode community in carrot production systems. Research was conducted at 2 sites in Michigan where cover crops were grown in fall 2014 preceding summer 2015 carrot production. Oats, 'Defender' radish, 'Dwarf Essex' rape, a mixture of oats and 'Defender' radish, and a fallow control were the treatments at each site. Nematode soil population densities were assessed: 1) during cover crop growth, 2) before planting carrots, 3) during carrot production, and 4) at carrot harvest. At site 1, root-lesion (*Pratylenchus* spp.) and stunt (*Tylenchorhynchus* spp.) nematodes were present at low densities (less than 25 nematodes/100 cm³ soil), but were not affected (ANOVA, $P > 0.05$) by cover crops. At site 2, root-lesion nematode densities were increased (ANOVA, $P < 0.05$) by 'Defender' radish compared to other cover crops or fallow control during cover crop growth and carrot production. Plant-parasitic nematode densities were not related to marketable carrot yield at either site suggesting other pathogens or environmental factors limited carrot yield more than plant-parasitic nematodes. At both sites, there were few short-term impacts of cover cropping on soil ecology based on the nematode community. At site 1, enrichment and structure indices were affected (ANOVA, $P < 0.05$) by cover crop treatments, but only at carrot harvest. Enrichment index was greater after oats-radish mixture or 'Dwarf Essex' rape than oats alone or fallow control. Structure index was greater after radish alone or 'Dwarf Essex' rape than oats alone. At site 2, bacterivore densities were increased by oats or radish cover crops compared to control, but only during carrot production. There were relatively few effects of cover cropping on the nematode community up to a year after cover crop growth suggesting that changes in the soil community following cover cropping may be gradual. Based on the results of this study, 'Dwarf Essex' rape and oats cover crops may have a neutral effect on management of root-lesion and stunt nematodes in Michigan carrot production, and 'Defender' radish may increase problems with root-lesion nematode.

PLANT-PARASITIC NEMATODES AND NEMATODE COMMUNITY COMPOSITION IN SELECTED MICHIGAN VEGETABLE FIELDS. **Grabau, Z.J.^{1,2}, B.P. Werling³, and H. Melakeberhan¹**. Michigan State University Department of Horticulture, East Lansing, MI, USA¹; University of Florida Department of Entomology & Nematology, Gainesville, FL, USA²; Michigan State University Extension-Oceana County, Hart, MI, USA³

Plant-parasitic nematodes can be serious pathogens of Michigan vegetable crops particularly root crops such as carrots. In contrast, free-living nematodes provide beneficial services such as nutrient cycling, residue decomposition, and pest/pathogen management. A survey of nematodes in selected Michigan vegetable was conducted in 2015 during crop production or following harvest. These fields were identified by growers or extension personnel as having potential plant-parasitic nematode infestation primarily based on high incidence of symptoms such as forked or stunted roots that reduce the market value of vegetables. Samples were collected from 32 fields in the major carrot-producing regions lower peninsula of Michigan. Fields included muck (11 fields) and mineral (21 fields) soils that were in carrot or other vegetable production. Plant-parasitic nematodes infested 30 of the 32 fields (94%). The plant-parasitic nematode genera detected included *Pratylenchus* (71% of fields sampled), *Meloidogyne* (41% of fields), *Paratylenchus* (26% of fields), and *Heterodera* (18% of fields). *Helicotylenchus*, *Paratrichodorus*, *Hoplolaimus*, and *Xiphinema* were each detected in one or two fields. Based on these results, plant-parasitic nematodes infestation is common in Michigan vegetable fields. Since carrots and other vegetable root crops are highly susceptible to plant-parasitic nematodes, this suggests economic loss due to plant-parasitic nematodes may be widespread in Michigan vegetable production. On average, in these fields, the nematode community was dominated by bacteria-feeding nematodes (67% mean relative abundance) while fungal-feeding (16% relative abundance) and plant-parasitic nematodes (14% relative abundance) constituted most of the rest of the community. Omnivores and predators (3%

relative abundance) represented a small proportion of the nematode community. This nematode community structure suggests Michigan vegetable fields have resource-rich, but relatively basic soil food webs.

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

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

GENETIC STRUCTURE AND EVOLUTION OF POTATO CYST NEMATODES POPULATIONS. **Grenier, E.¹, P.-Y. Véronneau², J. Lafond-Lapalme², J. Montarry¹, S. Fournet¹ and B. Mimee².** ¹INRA, Institute of Genetic, Environment and Plant Protection, Le Rheu, France, 35653, ²AAFC, St Jean-sur-Richelieu, QC, Canada, J3B 3E6.

To understand the mechanisms leading to the breakdown of plant resistance by potato cyst nematodes (PCN; *Globodera rostochiensis* and *G. pallida*), an understanding of the genetic structure and variation in populations and the processes that govern their evolution is required. Microsatellites and single nucleotide polymorphisms (SNPs) obtained by genotyping-by-sequencing (GBS) were used for phylogeographic and population genetic studies of PCN. The highest genetic diversity was consistently observed in populations from South America where these nematodes coevolved with their hosts. At a worldwide scale, results showed that, (i) both PCN species may share a single common introduction origin into Europe; (ii) the uplift of the Andes Mountains triggered a variety of adaptive biotic radiations representing a key factor in PCN evolutionary history. At a smaller spatial scale (regional, field), the genetic structure of different *Globodera* species showed that PCN exhibit much higher levels of gene flow at the intra-regional level compared to tobacco cyst nematode (*G. tabacum*), highlighting the importance of passive gene flow due to cultural practices in the dispersion and evolution of PCN populations. Additionally, most of the genetic variability observed at the field scale, or even regionally was already observed at the scale of a single plant within a field. Finally, as populations of *Globodera* differ in virulence against the various resistance sources used in potato varieties, it is of paramount importance to be able to identify pathotypes/variants or to be able to identify novel gene pool introductions. To this end, GBS has been used to identify SNPs to quickly distinguish PCN variants or groups that strongly differ in their development rates on a set of resistant potato cultivars.

PREPARATION OF A DIAGNOSTIC TOOL FOR *MELOIDOGYNE* SPECIES DIFFERENTIATION IN ALABAMA. **Groover, W.¹ and K. Lawrence¹.** ¹Department of Entomology and Plant Pathology, Auburn University, AL 36849.

Meloidogyne species identification is important for growers in the state of Alabama, because it helps in the decision of crop rotations. Depending on what species is present in a root-knot nematode infestation, a year to year crop rotation can be implemented as a means to help with control. It also helps growers determine if resistant varieties are needed based upon certain root-knot species levels. Currently, identification of *Meloidogyne* species for Auburn University is performed via a host-differential test. The host-differential test is a commonly used method for species identification. This test can take as long as forty-five to sixty days for successful species determination. A broader assay is currently in development to expedite the identification time to as quickly as a week. This process includes morphological measurements as well as the use of molecular techniques to provide an accurate depiction of what species are present. The molecular technique involves the use of a single *Meloidogyne* (root-knot) second stage juvenile that is ruptured in a droplet of water, and then added to a PCR mixture. Primers currently used screen for the following common *Meloidogyne* species: *M. incognita*, *M. arenaria*, *M. javanica*, *M. hapla*, *M. chitwoodi*, and *M. enterolobii*. Samples from known root-knot infested fields throughout the state of Alabama are currently being taken for analysis. The southern root-knot nematode, *M. incognita*, has been the most prevalent species identified to date. Peanut root-knot nematode, *M. arenaria*, has also been discovered on peanut fields in the southern part of the state. Each of these species has been identified via a host-differential test. For *M. incognita*, gall formation and nematode egg numbers were present on cotton, pepper, watermelon and tomato, showing the presence of *M. incognita* race 3. For the *M. arenaria* population, galls and nematode eggs were present on tobacco, pepper, watermelon, peanut, and tomato. This shows that the species present was *M. arenaria* race 1. Morphological measurements and features for adult males, females and juveniles were then taken of each of these species populations, and were found to fit the expected ranges for each relative species. *M. incognita* has

successfully been identified by a PCR technique using the primer set Inc-K14. *M. arenaria* has yet to be identified via molecular techniques. All primers screened so far for *M. arenaria* have failed to show amplification. The next steps in this research include continued work on developing a PCR technique for species differentiation, as well as sequencing several genetic regions in both populations to help further differentiate the species. Going forward, we hope to build a complete diagnostic assay that can positively identify all commonly found species of *Meloidogyne* as quickly, efficiently, and accurately as possible.

BERMUDAGRASS ROOT ROT DISEASE COMPLEX ASSOCIATED WITH *BELONOLAIMUS LONGICAUDATUS* AND *PYTHIUM* SPP. Gu, M.¹ and W.T. Crow¹. ¹Entomology & Nematology Dept. University of Florida, Gainesville, FL 32611, USA.

Pythium root rot disease is considered one of the major bermudagrass diseases on in Florida. In a previous nematicide greenhouse testing experiment, creeping bentgrass growing in pots inoculated with *Belonolaimus longicaudatus* easily acquired *Pythium* root rot. Based on the samples from the UF/IFAS Nematode Assay Lab, *Pythium aristosporum*, *P. catenulatum*, and *P. middletonii* were isolated from bermudagrass samples with high *B. longicaudatus* population density (over 25 *B. longicaudatus*/100 cm³ of soil). An experiment was conducted to determine if *B. longicaudatus* increases the incidence and severity of bermudagrass root rot caused by these *Pythium* spp. Each of these three *Pythium* isolates and ten *B. longicaudatus* were inoculated separately and in combination to bermudagrass planted in 10 ml pipette tips 4-week or 5-week after sprigging; an uninoculated control was included. Inoculated pipette tips were arranged in a completely randomized design with ten replications. Eight weeks after sprigging, five replications were destructively sampled to measure root necrosis, root length, and *B. longicaudatus* population density; the other five replications were plated to determine if the *Pythium* sp. had infected the roots.

EFFECTO DE NEMATÓXICOS ORGÁNICOS SOBRE LA EMERGENCIA DE HUEVOS Y MORTALIDAD DE JUVENILES DE MELOIDOGYNE SPP. EN CONDICIONES DE LABORATORIO. (EFFECT OF ORGANIC NEMATOXICS ON THE EMERGENCY OF EGGS AND MORTALITY OF JUVENILES OF MELOIDOGYNE SPP UNDER LABORATORY CONDITIONS). Guardia, H.¹ and C. Cedano². ¹AVIBIOL SAC, Calle Luis Arias Schreiber 215 Dpto 201. Miraflores-Lima. Perú. ² Universidad Nacional de Trujillo. Avenida Juan Pablo II s/n Trujillo- La Libertad, Perú.

Se evaluó el impacto de nematódicos orgánicos sobre la emergencia de huevos y mortalidad de juveniles de *Meloidogyne* spp., en condiciones de laboratorio, utilizando un diseño completamente al azar con diez repeticiones y cinco tratamientos: Testigo T0 (Sin Aplicación), T1 (Nemaquill: 1.5L/200L), T2 (Nemathor 1.5L/200L), T3 (Hunter: 400ml/200L) y T4 (Vydate: 500ml/200L). Los huevos y J2 tratados, fueron extraídos de raíces de apio (*Apium graveolens* L. var. Ventura) con gran cantidad de nódulos. La viabilidad de los huevos fue determinada en agua destilada estéril encontrándose que el promedio de emergencia natural de juveniles (J2) fue de 23.32%. Los productos en estudio fueron preparados en dilución en agua destilada estéril y en forma sólida en agar al 1% esterilizado y posteriormente fueron servidos en placa de Petri (15mL/placa). En cada placa se colocó 50 huevos ó 50 J2 según el caso. Cada placa se consideró como una unidad experimental. Los resultados mostraron que T2 inhibió totalmente la emergencia y también causó el 100% de mortalidad de J2, tanto en forma líquida como sólida. T4 mostró 1.03% (líquido) y 1.05% (sólido) de emergencia y 100% de mortalidad; T1 y T3 mostraron emergencia de 15.17 y 35.35% y mortalidad de 0 y 5.57% respectivamente, en líquido; y en sólido produjeron emergencia de 15.39% y 34.36% y mortalidad de 0 y 6.23%, respectivamente.

NEW COMPOUNDS AND CHEMISTRIES FOR CONTROLLING NEMATODES, 2013-2015. Hafez, S.L. University of Idaho Parma REC, 29603 UofI Lane, Parma Idaho 83660.

Potato and sugar beet are the major crops that impact agricultural economy of the state of Idaho, USA. The major nematode pests on potato are *Meloidogyne chitwoodi*, *Pratylenchus penetrans*, *Pratylenchus neglectus*, *Paratrichodorus* sp., *Ditylenchus dipsaci* and the recently discovered *Globodera pallida* and all can cause considerable damage to tuber yield and dramatic economic loss. Sugar beet cyst nematode *Heterodera schachtii* is the predominant nematode pest causing yield declines and severe economic damage to the region. Management of any plant parasitic nematode is highly desirable. Chemical management tactics or programs and the recent development of non-chemical practices established by the University of Idaho Nematology Lab can considerably reduce nematode damage potential to potato and sugar beet crops as well as some onion and corn crops. Most of the combination practices can be commercially adapted by growers and are economically viable. New compounds and chemistries were tested and evaluated for efficacy as nematicides under field conditions at the University of Idaho Parma Research and Extension Center in Parma, Idaho, USA. Products were tested for efficacy in potato on root knot and lesion nematodes, sugar beet on sugar beet cyst nematode, onion and/or corn on lesion nematode. Products were applied either as a stand-alone application, incorporated in standard Vydate programs, combined with Vapam fumigations or integrated into multiple product and application programs. Experimental compounds and chemistries consisted of granular or liquid Nimitz at varying rates in potato with a Vydate program or as a stand-alone in potato and sugar beet, Movento 240 SC finishing off application programs in various potato or onion programs or stand-alone timing applications in sugar beet, BCS-CT#, Varnimo WSP or VBC# as potato stand-alone applications, BCS-CS# in liquid or granular form as a stand-alone or in a Vydate program in potatoes, BCS-AR# incorporated in all experimental crop's various programs, Luna Tranquility or SP#13 assimilated into various potato programs, Pasteria seed-treatment as a potato

and sugar beet seed-treatment program, Q8U80 in liquid or granular form in various programs in potato and sugar beet and as a stand-alone in onion, BioAct and SP#66 in potato as a stand-alone or in various programs, and Propulse integrated in sugar beet and corn programs. 93% of all experimental potato treatment programs resulted in an increase in either harvested or marketable yield. 80% of all experimental sugar beet treatments were effective in increasing total beet yield. 65% of onion and corn treatment programs provided an increase in total yield. Overall, 86% of all treatments containing at least one new compound or chemistry resulted in an increase in yield. Most new compounds and chemistries can affect nematode populations and reduce yield damages or losses. However, multiple product and application programs, Vapam and Vydate programs tended to produce higher yields and better results than stand-alone product treatments. It is also to be noted that in some cases phytotoxicity complications have negated the effects of nematode population reductions. New experiments and formulation changes have been proposed and are currently underway to address these concerns. The testing of new compounds and chemistries as well as the experimentation with non-chemical methods can benefit growers.

CHEMICAL MANAGEMENT PRACTICES FOR THE MANAGEMENT OF *MELOIDOGYNE CHITWOODI* ON POTATO IN IDAHO, USA. **Hafez, S.¹ and P. Sundararaj²**. ¹U of I Parma REC, 29603 U of I Lane, Parma, ID 83660, USA; ²Bharatiar University, Coimbatore-641046, Tamilnadu, India.

Two field experiments were conducted to determine the efficacy of Telone II, Vapam, Mocap and Vydate alone or in combination with either Tellone II (Experiment I) or Vapam (Experiment II) fumigations for the control of Columbia root-knot nematode in potato. Both experiments were carried out in randomized complete block design with five replications and one untreated control treatment in a silt loam field naturally infested with *Melodogyne chitwoodi* at a population average of 5.4/cc soil. Telone II and Vapam were applied pre-planting with a commercial applicator by ripper and fumigation bar to a depth of 16-18 and 12-14 inches respectively. Mocap was also applied pre-planting and was surface broadcasted using a handheld CO₂ powered plot sprayer and then incorporated. Vydate was applied at planting using a CO₂ powered plot sprayer mounted on the potato planter. Foliar applications of Vydate began at 1700 degree days and were repeated in 14 day intervals for a total of 7 applications. Potatoes were harvested from the middle two rows of each plot at maturity and total, infected and percent infected yields were recorded. In Experiment I, Telone II, Vapam or Telone II + Vapam significantly reduced infected potato yield (0.2-1.0%) as compared to other treatments. In Experiment II, Telone II, Vapam, and Telone II + Vapam treatments resulted in the lowest percent of infected yield as compared to Vydate and Mocap treatments. These studies indicate that applications of 37.5 gal/A Vapam is effective in controlling *M. chitwoodi* in potato.

LESION NEMATODE AND VERTICILLIUM WILT INTERACTIONS IN GREENHOUSE MINT. **Hafez, S.L. and C. Sevy**. U of I Parma REC, 296032 Uof I Lane, Parma, Idaho 83660.

Little is known about lesion nematode interactions in mint or when nematodes are combined with specific fungi. An experiment to determine the interactions between the common mint pests, *Verticillium dahliae*, *Pratylenchus penetrans* and *Pratylenchus negelectus* was conducted at the University of Idaho Research and Extension Center Parma, Idaho. Sterile soil was inoculated with 20 colony forming units of *V. dahliae* and/or 4 nematodes/cc soil for either lesion species. After approximately six weeks of growth hay was harvested just above soil level and weighed before being dried at 120° for 48 hours. Dry weights were recorded for statistical analysis and mint was allowed to regrow for another six weeks before a second harvest. The experiment was repeated for consistency shortly after the conclusion of the first experiment. Results showed that *V. dahliae* alone could reduce dry hay yields up to 50%, whereas either species of lesion nematode could only reduce day hay yield up to 44% with an average of 30% reduction over two experiments. A combination of *V. dahliae* and *P. negelectus* results in a mas dry hay reduction of 74% with an average of a 60% reduction between the two experiments. *V. dahliae* combined with *P. penetrans* results in a maximum reduction of 98% with an average loss of 96% of the day hay yield. The combination of all three pests yielded almost no hay with a maximum loss of 99% of hay and an average day hay yield reduction of 98%. All three pests can be considered highly pathogenic and are capable of considerable damage to mint crops alone. However, when combined pathogenicity dramatically increased with the addition of *V. dahliae* to either lesion nematode species with *P. penetrans* having a significantly higher pathogenicity as compared to *P. negelectus*.

IS THE STEM AND SEED NEMATODE OF CREEPING THISTLE *DITYLENCHUS WEISCHERI* A PEST OF YELLOW PEA? **Hajihassani, A.¹, M. Tenuta¹, and R.H. Gulden²**. ¹Department of Soil Science, ²Department of Plant Science, University of Manitoba, Winnipeg, Manitoba, R3T 2N2, Canada.

Some species of *Ditylenchus* are destructive pest nematodes of agricultural crops. We recently reported that the recently described species, *D. weischeri*, occurred on creeping thistle (*Cirsium arvense* L.) in Manitoba and Saskatchewan. This stem and seed nematode of creeping thistle is closely related to and likely previously reported as *D. dipsaci* on the Canadian Prairies. The host preferences of *D. weischeri* are unknown. Therefore, we screened major pulse and non-pulse crops of the Canadian Prairies as being hosts for *D. weischeri* and *D. dipsaci* under greenhouse conditions. *Ditylenchus weischeri* reproduced aggressively on creeping thistle. Out of five varieties of yellow pea tested, Agassiz and Golden were poor hosts for the nematode having a reproductive factor (ratio of final to initial population) of slightly > 1, the other varieties were not

hosts. Common bean, chickpea, lentil, spring wheat, canola, and garlic were not hosts for *D. weischeri*. In contrast, *D. dipsaci* reproduced well on yellow pea, common bean, chickpea and lentil but not on creeping thistle, wheat and canola. *Ditylenchus weischeri* was not a seed-borne parasite of yellow pea, unlike, *D. dipsaci* which was recovered from seeds of the grown yellow pea. Conversely *D. weischeri* and not *D. dipsaci* was recovered from creeping thistle seeds. The development and reproduction of the nematodes were investigated in a growth chamber at temperatures of 17, 22 and 27 °C. *Ditylenchus weischeri* did not complete a generation at 17 and 22 °C but at 27 °C the nematode generation time took a minimum of 30 days. In contrast, the minimum generation time for *D. dipsaci* was 24, 18 and 22 days at 17, 22 and 27 °C, respectively. The reproduction of *D. weischeri* on yellow pea is unlikely in the Canadian Prairies where mean daily air temperatures of 27 °C are rare and not sustained. Under field microplot conditions, yellow pea height, above-ground biomass, pod length, and grain yield were not significantly affected by addition numbers (100-3200 nematodes/plant) of *D. weischeri* to two yellow pea varieties. At harvest, the total number of recovered nematodes per plant was not significantly different than that added. There was a concern that since *D. weischeri* was morphologically and genetically related to *D. dipsaci*, it may be a crop pest on the Prairies. Our results demonstrate that the host preference of *D. dipsaci* differs from *D. weischeri* with creeping thistle being a suitable host. However, more research is needed regarding the ability of *D. weischeri* to parasitize crops at high temperatures.

FIRST REPORT OF STEM AND BULB NEMATODE, *DITYLENCHUS DIPSACI*, INFESTING GARLIC IN MANITOBA, CANADA. **Hajihassani, A. and M. Tenuta.** Department of Soil Science, University of Manitoba, Winnipeg, Manitoba, R3T 2N2 Canada.

The stem and bulb nematode, *Ditylenchus dipsaci*, causes serious yield and quality loss to many crops such as garlic, alfalfa, common bean, pea and many ornamental plants. In Canada, this nematode is a common parasite of garlic in Ontario and more recently Quebec. In summer 2015, diseased garlic stems and bulbs were received from two growers in Southern Manitoba having the previous fall planted seed pieces from Ontario. Plants were stunted and exhibited malformation and twisting, blistering and dieback of leaves. The bulbs were soft and spongy with few roots. Further, conidia of *Fusarium* were recovered from bulbs. Recovered female and male nematodes were morphologically (body length, stylet length, maximum body width, body width at anus, vulva length, spicule length, tail length) similar to *D. dipsaci*. Individual adults were extracted for DNA extraction and a portion of the hsp90 gene amplified using primer set U831 (5'-AAYAARACMAAGCCNTYTGGAC-3') and Dipsaci_hsp90R (5'-GWGTTAWATAACTTGGTCRGC-3') specific for *D. dipsaci*. Amplification resulted in confirmation of identity as *D. dipsaci*. This nematode pest has not been previously reported in Manitoba. We believe that the nematode has been introduced into Manitoba by importing infested seed bulbs of garlic from Ontario. Although garlic is not a major crop in Manitoba, steps should be taken to prevent presence in garlic fields and transmission to commercially important crops such as common bean, field pea, and alfalfa.

MONOXENIC REARING OF *DITYLENCHUS WEISCHERI* AND *D. DIPSACI* ON CALLUSED ALFALFA TISSUE AND CARROT DISKS. **Hajihassani, A. and M. Tenuta.** Department of Soil Science, University of Manitoba, Winnipeg, Manitoba, R3T 2N2 Canada.

Ditylenchus weischeri was recently reported in the provinces of Manitoba and Saskatchewan, Canada. A population of *D. weischeri* from creeping thistle (*Cirsium arvense* L.) in Manitoba and *D. dipsaci* from garlic (*Allium cepa* L.) in Ontario were examined in the current study for potential to be reared on callused carrot (*Daucus carota* subsp. *sativus*) disk with no medium, alfalfa (*Medicago sativa* L.) and creeping thistle callus tissues, and pure cultures of eight fungal species, *Botrytis cinerea*, *Fusarium solani*, *Rhizoctonia solani*, *Verticillium dahliae*, *Sclerotinia sclerotiorum*, *Cladosporium cucumerinum*, *Colletotrichum gloeosporioides*, and *Chaetomium* spp. (isolated from creeping thistle leaves). Nematodes were surface sterilized for 15-18 hours in streptomycin sulfate solution (4000 mg/L), and then for 10 min in mercuric chloride (1000 mg/L) and rinsed with sterile distilled water. Eighty fourth-stages juveniles were then added to the callus produced on the carrot disks, alfalfa and creeping thistle tissues as well as plates of fungal cultures. *Ditylenchus weischeri* and *D. dipsaci* could not be reared on the fungal isolates as well as the callus of creeping thistle. In contrast to *D. weischeri*, *D. dipsaci* was successfully reared on alfalfa tissue callus after 90 days at 23±1°C, with an increase of 67 times the number of nematodes initially added. In callused carrot disks, an increase of 54 and 244 times the addition density of 80 nematodes was obtained for *D. weischeri* and *D. dipsaci*, respectively. The results indicate monoxenic rearing of *D. weischeri* using callused carrot disks can be used to provide large numbers of individuals for use in studies such as those considering plant host preference.

SOUTHERN ROOT-KNOT NEMATODE (*MELOIDOGYNE INCOGNITA* RACE 3) SUSCEPTIBILITY OF THIRTEEN CULTIVARS OF TURMERIC (*CURCUMA* SPP.). **Hall, M.¹, K.S. Lawrence¹, D. Shannon², T. Gonzalez², W.L. Groover¹, and J.A. Luangkhot¹.** ¹Dept. of Entomology and Plant Pathology, Auburn University, Auburn, AL 36849, ²Department of Crop, Soil, and Environmental Sciences, Auburn University, AL 36849.

Turmeric (*Curcuma* spp.), a spice crop native to India, has experienced a dramatic increase in demand due to its anti-inflammatory and other health-promoting properties. Turmeric is currently being evaluated as a potential cash crop for Alabama and other Southeastern U.S. states. Root-knot nematode (*Meloidogyne* spp.) negatively affects turmeric production

systems, causing significant losses to production each year. Because *Meloidogyne* spp. are classified as an endemic pest throughout regions of Alabama, cultivar selection for tolerance or resistance to the root-knot nematode will be a key factor in establishing successful turmeric production. Thirteen cultivars of turmeric cultivated in the Auburn University Medicinal Plant Garden were evaluated for root-knot nematode susceptibility; a host differential test indicates the *Meloidogyne* species present is *M. incognita* race 3, and PCR sequencing will be conducted to confirm this observation. The thirteen turmeric cultivar plots were replicated four times, and soil samples were taken from each plot. *M. incognita* were extracted from the soil using the sucrose centrifugation-flotation method and enumerated. Data was analyzed using SAS 9.4 PROC GLIMMIX and LS-means compared by the Tukey-Kramer method with significance level of $\alpha \leq 0.05$. All thirteen cultivars supported *M. incognita* numbers, ranging in LS-means from 64-1,285 juveniles per 150cc of soil. *Curcuma longa* 5 supported the maximum *M. incognita* population (1,285 juveniles per 150cc of soil) of all thirteen cultivars assessed ($P \leq 0.05$); all other cultivars sustained significantly lower numbers of *M. incognita* than *Curcuma longa* 5, ranging from 64-393 juveniles per 150cc of soil. Greenhouse screening of the rhizomes from the thirteen cultivars will be conducted during the 2016-2017 seasons to confirm the field observations of southern root-knot nematode susceptibility as well as to evaluate *M. incognita* race 3 effects on growth parameters and rhizome yield. Ultimately, identification of *M. incognita* race 3 susceptible cultivars of turmeric will provide crucial information to potential growers in southern root-knot nematode infested areas of Alabama and similar growing regions.

THE ROLE OF THE NEUROTRANSMITTER SEROTONIN AND GABA IN PLANT-PARASITIC NEMATODES.

Han, Z., S. Boas and N.E. Schroeder. Department of Crop Sciences, University of Illinois, Urbana, IL 61801.

While the habitats and behaviors of nematodes are diverse, the nervous system of nematodes is often considered highly conserved. We recently found a surprising amount of variation in the neuroanatomy both within and among several nematode clades. Plant-parasitic nematodes (PPNs) have distinct anatomies and behaviors compared to the free-living nematode *Caenorhabditis elegans*. How individual behaviors are controlled in PPNs is unclear. Several neurotransmitters are important for regulating behaviors in *C. elegans*. The neurotransmitter serotonin regulates feeding and mating behaviors, while the neurotransmitter GABA regulates foraging, defecation and locomotion in *C. elegans*. We have detected the serotonergic cells in the root lesion nematode *Pratylenchus penetrans* using immunohistochemistry. The pattern of serotonergic staining was similar to *C. elegans*. We found serotonergic cells in the head region that are likely homologs to the *C. elegans* NSM and ADF in the esophagus and amphids, respectively. We also found staining in several neurons surrounding the vulva in adult *P. penetrans* females and several neurons in the male ventral nerve cord. We found that application of exogenous serotonin causes spicule eversion in the males, vulva contraction in the females, and an increased stylet thrusting rate in all developmental stages. We have also cloned the genes encoding the key enzymes controlling the biosynthesis of serotonin (*hg-tpH-1*) and GABA (*hg-unc-25*) from the soybean cyst nematode *Heterodera glycines*. *hg-TPH-1* and *hg-UNC-25* are 65% and 76% identical, respectively, to the *C. elegans* orthologs. We will confirm the gene function of the *hg-unc-25* by using heterologous rescue in the *C. elegans unc-25* mutant. We will use in-situ hybridization to determine the expression pattern of *hg-unc-25*.

MORPHOLOGICAL DIAGNOSTICS OF POTATO CYST NEMATODES (*GLOBODERA* SPP.) **Handoo, Z.A.¹, L.K. Carta¹, A.M. Skantar¹, and D.J. Chitwood¹.** ¹Nematology Laboratory, USDA, ARS, Building 010A, BARC-West, 10300 Baltimore Avenue, Beltsville, MD 20705.

Potato cyst nematodes (*Globodera* species) are economically important pests of solanaceous crops including tomato, eggplant and especially potato. The potato cyst nematodes (PCN) *Globodera rostochiensis* and *G. pallida* are regulated pathogens of potato, an extremely important commercial crop in the United States with over 1 million acres planted at a value of nearly \$3.9 billion. Another PCN (*G. ellingtonae*), recently described from Oregon in the United States, triggered a quarantine of the location from where it was isolated to prevent its spread. Although PCN can reduce crop yields worldwide, methods for identification are often difficult to implement due to variations noted in life stages among various populations. We summarize the diagnostic morphological features for distinguishing the above three PCN species on potato and compare them with the closely related species complex, the tobacco cyst nematode (TCN) or *G. tabacum* complex and other round cyst nematode species. In addition, photomicrographs and drawings illustrating key diagnostic characters of all life stages (cysts, females, males and juveniles) are provided together with information on host and geographic distribution. A brief summary of comparative diagnostic characters of all life stages is presented in a tabular form, along with general information on the economic impact of PCN, TCN and other closely related species.

THE EFFECTS OF FLUOPYRAM ON NEMATODES. **Heiken, J.¹, M.R. Schwarz², E.L. Davis¹, and C.H. Opperman¹.** ¹Department of Plant Pathology, North Carolina State University, Raleigh, NC 27695; ²Bayer CropScience LP, P.O. Box 12014, Research Triangle Park, NC 27709.

In recent years, chemical control options for plant-parasitic nematodes have become increasingly limited due to both increasing restrictions on use and deregistration of existing compounds. The lack of safe and effective nematicides for commercial use against root parasitic nematodes is a critical problem faced by farmers with few options for alternative

management schemes. The development of new compounds to combat devastating nematode infections is urgently needed. In this work, fluopyram, the active ingredient in the fungicide Luna Privilege™, was tested for its nematocidal properties. Fluopyram works as an inhibitor of succinate dehydrogenase, an enzyme essential in the TCA cycle and electron transport chain. Fluopyram had been previously observed to have potential nematode suppressive effects in field trials, and the effects of fluopyram on nematodes were further studied *in vitro* in our experiments. We first examined the efficacy of technical grade fluopyram on the viability of mixed life stages of *Caenorhabditis elegans* and determined an ED50 of 11.4 ppm. *C. elegans* mutants VC294 with altered succinate dehydrogenase activity were treated with fluopyram and showed greater sensitivity than wild type *C. elegans* with an ED50 of 4.31 ppm. Experiments with second-stage juveniles (J2) of *Meloidogyne incognita*, and *Heterodera glycines* revealed a similar sensitivity in viability to fluopyram. Recovery assays were performed to determine whether the effects of fluopyram are reversible. When exposed to fluopyram at 10 ppm for an hour, *C. elegans* showed no sign of recovery in viability. Hatching assays were performed with *M. incognita* and *H. glycines* eggs to determine potential effects of fluopyram on hatch of infective J2. *M. incognita* eggs hatched in fluopyram concentrations as low as 25 ppm showed a decrease in hatch rate from 76.9% hatch in water control to 4.15% hatch in 25 ppm fluopyram. The results suggest that the effects of fluopyram on nematodes are truly nematocidal to all nematode life stages. The effects of fluopyram applications in soil on root-knot and cyst nematode infection of tomato and soybean plants, respectively, and how organic matter content of soil may influence the efficacy of fluopyram, will be the subject of subsequent investigations.

EFFECT OF KLAMIC® ON GROWTH STIMULATION OF PLANTAIN AND BANANA IN-VITRO PLANTS. Hidalgo-Díaz Leopoldo¹, M. A. Hernández¹, J. Arévalo¹, and D. Marrero². ¹Centro Nacional de Sanidad Agropecuaria (CENSA), carretera de Jamaica y Autopista Nacional, Apartado 10, San José de las Lajas, Mayabeque, Cuba, ²Biofábrica. Finca el Llano, carretera de Jamaica km 2 ½, San José de las Lajas, Mayabeque, Cuba.

The nematophagous fungus *Pochonia chlamydosporia* (Clavicipitacea) is becoming one of the most studied biocontrol agents of plant endo-parasitic nematodes. The fungus is a parasite of nematode eggs but occurs as a saprophyte in soils. In the rhizosphere, the fungus colonizes the roots of host plants and some *Pochonia* species have an endophytic behavior that may be beneficial to the host plant's defense against other soil borne pathogens. In Cuba, the native strain IMI SD 187 of *P. chlamydosporia* var. *catenulata* was selected and developed as a biological control product (KlamiC), which is registered for use against root-knot nematodes in Cuba, Nicaragua and Panama. The objective of this investigation was to determine the endophytic activity of the fungus and its effect on plant growth promotion of banana and plantain *in vitro* plants. The CENSA^{3/4}, Pisang Ceilan, FHIA-01 and FHIA-18 cultivars were used in the *ex vitro* adaptation area, in polypropylene trays and black polyethylene bags, containing a bovine compost substrate. Two applications were made with KlamiC® at a concentration of 5.6×10^5 chlamydospores per plant⁻¹. The vegetative growth variables and the substrate and root colonization by the fungus were measured at the end of the experiment. A randomized experimental design was used, with 70 repetitions per treatment, KlamiC® and the absolute control without the product, for each cultivar. The data were analyzed and compared statistically. A significant increment was obtained in all the variables of the growth of the plants treated with KlamiC® in comparison with the plants not treated. For all the cultivars evaluated, the substrate and rhizosphere colonization by the fungus was confirmed in the treatments with KlamiC®. The smallest percentage of endophytic colonization was reached in the FHIA-18 cultivar with 4.15%, the others one was between 16 and 21%. These results demonstrate the potential of KlamiC® as a growth promotion product and suggest the evaluation of different methods and frequency of application during the production of banana and plantain *in vitro* plants and its behavior in the field condition.

INVESTIGATION OF COEVOLUTION BETWEEN XIPHINEMA SPP. AND BACTERIAL ENDOSYMBIONTS. Howe, D.K.¹, D.M. Tom¹, M. Smith¹, A.M.V. Brown¹, A.B. Peetz², I.A. Zasada², and D.R. Denver¹. ¹Department of Integrative Biology, Oregon State University, Corvallis, OR 97331; ²USDA-ARS Horticultural Crops Research Laboratory, Corvallis, OR 97330.

Plant parasitism is widespread within nematodes – 15% of known species parasitizing plants across a broad host range. At least three independent origins of plant parasitism are found within Phylum Nematoda, making them important pests in a wide range of agricultural systems. This study focused on the processes of evolution and symbiosis in *Xiphinema americanum*-group nematodes. The *X. americanum*-group nematodes are known to harbor the obligate bacterial endosymbiont *Xiphinematobacter* in the ovaries and gut, where it likely serves as a nutritional mutualist. Our study compared molecular phylogenetic congruency between host and symbiont for dozens of *X. americanum*-group populations and related *Xiphinema* species from 16 sites in North America, including nine US states and one Canadian province. We PCR amplified one mitochondrial DNA region (mtDNA) from individual *Xiphinema* species and two loci from the endosymbiont *Xiphinematobacter*: a segment of the 16S rRNA gene (slow-evolving) and a segment of the NAD synthetase protein-coding gene (fast-evolving). The amplicons were sequenced and we used Maximum Likelihood phylogenetic analyses to investigate evolutionary relationships, and the potential for coevolution between hosts and symbionts. Phylogenetic trees resulting from nematode mtDNA and symbiont DNA revealed patterns of widespread coevolution; mtDNA and 16S loci had three congruent monophyletic clades, while mtDNA and NAD loci provided finer resolution, producing six congruent monophyletic

clades. These results were consistent with strict vertical transmission and obligate association between the nematode and endosymbiont.

THE DISTRIBUTION AND MANAGEMENT PRACTICES OF *HETERODERA GLYCINES* IN MISSOURI IN 2015. **Howland, A.¹, M. Nathan¹, and M.G. Mitchum¹.** ¹Division of Plant Sciences, University of Missouri, Columbia, MO 65211.

One of the most economically devastating pathogens facing soybean producers is, the soybean cyst nematode (SCN), *Heterodera glycines*, costing U.S. soybean producers an estimated \$1.2 billion annually. Determining the presence and virulence phenotypes of SCN is essential for devising management strategies implementing the use of resistant cultivars. A statewide survey was conducted to determine the distribution and virulence phenotypes of SCN in Missouri and to assess grower awareness during 2015. One hundred soil samples from fields representing eight geographic regions with high soybean production were collected and processed for SCN egg counts. In all, 89% of the samples tested positive for SCN with 74% of these at >500 eggs/250 cm³ soil. Despite this, only 34% of farmers were aware of a SCN problem, and 57% reported that they had never sampled their fields. Modified HG type tests were conducted on populations from 20 samples. All populations had a female index (FI) above 10% on PI88788, the source of resistance found in >90% of SCN-resistant cultivars, with 58% of those tests having an FI over 50%. Sixty-seven percent of populations had a FI above 10% on Peking, another common source of resistance, but few could reproduce on PI90763 and none on PI437654. The greatest genetic variability in SCN populations was found in the southeast region of Missouri, consistent with the diversity of resistant sources (Peking, PI88788, and Hartwig type) planted in this part of the state. Despite more than 68% of farmers reporting that they plant SCN resistant varieties, only 25% were aware of the source of resistance and how SCN adapts to it. Our survey highlights the importance of grower awareness and the need to increase the genetic diversity of SCN resistance in commercially available SCN resistant soybean cultivars to minimize yield loss due to SCN into the future.

ETHYLENE RESPONSE PATHWAY MODULATES ATTRACTIVENESS OF PLANT ROOTS TO *HETERODERA GLYCINES*. **Hu, Y.¹, J. You¹, C. Li¹, V.M. Williamson², and C. Wang¹.** ¹Key Laboratory of Mollisols Agroecology, Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences, Harbin 150081, China, ²Plant Pathology Dept., University of California, Davis, CA 95616, USA.

Plant parasitic nematodes respond to root exudates to localize host roots. The ethylene (ET) response pathway has been reported to negatively modulate host attractiveness to the root-knot nematode *Meloidogyne hapla* but to increase attractiveness for the sugar beet cyst nematode *Heterodera schachtii*. Our preliminary studies showed second stage juveniles of *Heterodera glycines*, the soybean cyst nematode (SCN), quickly migrated to soybean roots when roots were put into Pluronic F-127 gel mixed with nematodes. Soybean roots treated with the ET-synthesis inhibitor aminoethoxyvinylglycine (AVG) were more attractive to RKN than untreated roots. Exogenous application of AVG resulted in a significant increase in the number of nematodes penetrated into soybean roots at 6 h after starting the attraction assay. The effect of the ET response pathway on SCN attraction to plant was further investigated in the non-host plant *Arabidopsis*. Exogenous application of AVG caused a significant increase in nematode numbers touching the *Arabidopsis* root tips. Moreover, *Arabidopsis* ET insensitive mutants (*ein2*, *ein2-1*, *ein2-5*, *ein3-1*, *ein5-1*, *ein6*, and *eil1-1ein3-1*) were much more attractive than wild-type plants. Conversely, the constitutive triple-response mutant *ctr1-1* and the ethylene-overproducing mutants (*eto1-2* and *eto3*) were less attractive to SCN. In contrast, ET receptor mutant *ein4-1* attracted more SCN than the wild-type plants. However, there are no significant differences in attractiveness between other gain-of-function ET receptor mutants (*etr1-3*, *etr1-7* and *ers1-3*) and the wild-type. We also found that ET signaling was activated in *Arabidopsis* in early response to SCN infection through detection of the expression of ET reporter construct, *EBS::GUS*. Strong GUS expression was observed in infection sites and surrounding cells at 6, 12 and 24 h after the starting assay. These results suggest that an active ethylene signaling pathway reduces attractiveness of plant roots to SCN in a similar way as to root-knot nematodes, but opposite to the results reported for *H. schachtii*.

IDENTIFICATION OF *MELOIDOGYNE* SPP. ON POTATO SEED TUBERS FROM YUNNAN PROVINCE IN CHINA. **Hu, Y.¹, C. Li¹, C. Hua¹, Y. Mao^{1,2}, and C. Wang¹.** ¹Key Laboratory of Mollisols Agroecology, Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences, Harbin 150081, China, ²Keshan Branch of Heilongjiang Academy of Agricultural Sciences, Keshan 161606, China.

Root-knot nematodes (*Meloidogyne* spp.) are among the most economically important plant-parasitic nematodes on many crops worldwide, including potato (*Solanum tuberosum*). However, little is known about the occurrence of root-knot nematodes on potato in China. In 2014, we received potato seed tubers from Yunnan Province for disease identification. The seed tubers were very small and deformed, with blisters on the surface. Within the vascular ring, there were brown spots where females and eggs of root-knot nematode were clearly seen. The morphological characteristics of females and second-stage juveniles matched the description of *M. javanica*. The intergenic region (IGS) within ribosomal DNA was amplified with primers 194/195 (TTAACTTGCCAGATCGGACG/TCTAATGAGCCGTACGC) and sequenced. The sequences obtained were 799 bp and

exhibited 99% identity with the sequences of *M. javanica* (KC287205, JX987322, and KC287206). For further confirmation of whether this was a single species or mixed species of root-knot nematodes, five PCR-specific primers for reported *Meloidogyne* spp. in Yunnan Province were used to amplify single nematodes and pooled nematodes. The specific primer pairs for *M. incognita* are Mi-F/Mi R (GGGATGTGTAATGCTCCTG/CCCGCTACACCCTCAACTTC), Fjav/Rjav (ACGCTAGAATTCGACCCTGG/GGTACCAGAAGCAGCCATGC) for *M. javanica*, Mh-F/Mh-R (TGACGGCGGTGAGTGCGA/TGACGGCGGTACCTCATAG) for *M. hapla*, Ma-F/Ma-(TCGGCGATAGAGGTAATGAC/TCGGCGATAGACTACAACCT) for *M. arenaria*, and Me-F/Me-R (AACTTTTGTGAAAGTGCCGCTG/TCAGTTCAGGCAGGATCAACC) for *M. enterolobii*. The electrophoresis results showed a bright band (~517 bp) for single nematodes and pooled nematodes only in the lane with the *M. javanica* specific primers. No product was found with other primer pairs. Based on its morphological and molecular characteristics, the pathogen was identified as *M. javanica*. In the greenhouse, plants of one of the major commercial potato cultivars in China were inoculated with the identified *M. javanica*. Thirty-five days after inoculation, galling and egg masses were detected on the roots, and blisters were found on the tubers which were similar to those observed in the original infected tubers. An increase in potato-growing area and spread of root-knot nematodes would make *M. javanica* a potential threat to potato production in China. Further research to evaluate current commercial cultivars for resistance and to develop resistant potato varieties would be an effective strategy for *M. javanica* management on potato.

JASMONATE SIGNALING INDUCES DEFENSE AGAINST *MELOIDOGYNE HAPLA* IN SOYBEAN. Hu, Y., J. You, C. Li, and C. Wang. ¹Key Laboratory of Mollisols Agroecology, Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences, Harbin 150081, China.

Root-knot nematodes (RKN, *Meloidogyne* spp.) are among the most important nematode parasites on soybean (*Glycine max*). While it is known that phytohormones play important roles in plant defense against plant-parasitic nematodes, the role of jasmonate (JA) in defense against RKN in soybean was unknown. To investigate whether JA plays a role in susceptibility/resistance in soybean, we compared two commercial soybean cultivars, Dongsheng 1 and Suinong 14, which are susceptible and resistant, respectively, to *M. hapla*. At 3 and 7 days after RKN infection, there were no significant differences between the cultivars in mean number of nematodes inside roots, but delayed development and production of fewer females, egg masses and eggs were observed in Suinong 14 compared to Dongsheng 1. Quantitative reverse transcription (qRT)-PCR analysis showed that the expression of genes involved in JA synthesis or signaling was significantly induced in both susceptible and resistant roots at 12 hours and 24 hours after inoculation. Exogenous foliar MeJA application to susceptible Dongsheng 1 triggered induced-resistance against *M. hapla* seen as lower number of nematode galls, egg masses or eggs and retardation of nematode development. In contrast, these indexes were not further decreased in resistant Suinong 14 with MeJA treatment. The activation of JA synthesis genes (*LOX*, *AOS1* and *AOS2*) by nematode invasion was slightly lower in MeJA-treated plants compared to untreated plants at 3 days post-inoculation, implying that JA might accumulate beyond an optimal level and cause feedback inhibition of the JA synthesis pathway during the systemic acquired resistance response. In addition, nematode infection also altered the expression pattern of marker genes in salicylic acid (pathogenesis-related genes *PR-1*, *PR-5*, *PR-10*) and ethylene (ethylene response factor, *ERF1*) pathway in the roots of both MeJA-treated and untreated susceptible plants. The data presented in this study indicate that JA signaling was activated in both susceptible and resistant soybean cultivars in the early stage of the RKN infection, and that exogenous foliar JA application to susceptible soybean plants activated defense against RKN.

PHYLOGENETIC RELATIONSHIP OF *MELOIDOGYNE SALASI* WITH OTHER RICE-PARASITIZING *MELOIDOGYNE* SPECIES. Humphreys-Pereira, D.A.¹, L.R. Arriola de², R. Sandoval-Ruiz¹, L. Gómez-Alpizar², and L. Flores-Chaves¹. ¹Laboratory of Nematology-CIPROC, University of Costa Rica, 2060 San Pedro, Costa Rica, ²Plant Biotechnology Laboratory-CIA, University of Costa Rica, 2060 San Pedro, Costa Rica.

Root-knot nematodes (*Meloidogyne* spp.) are an important pest in the majority of rice production areas world-wide. Information about the distribution and genetic diversity of *Meloidogyne* species associated with rice in Costa Rica is unknown. Roots and soil samples of rice were collected from the Atlantic (Huetar Atlántica), Southern (Brunca) and Central Pacific (Pacífico Central) regions of Costa Rica. A group of samples were also collected from the area known as La Cuesta, where *M. salasi* was originally found and described as a new species in the early 80s. Most of rice plants infected with *Meloidogyne* sp. showed symptoms of stunting, yellowing and root galls. *Meloidogyne* spp. populations extracted from rice were characterized using the nuclear marker D2-D3 expansion segment of the large subunit 28S rDNA and the mitochondrial marker between *cox2* and *16S rRNA* genes. Two more markers (*18S* and the ITS region) are also currently being evaluated. Sequences were aligned using the program MUSCLE and the best-fit model of nucleotide substitution for each molecular marker was selected based on the Bayesian information criterion (BIC). The phylogenetic relationship of *M. salasi* with other *Meloidogyne* species was estimated using Bayesian Inference and Maximum likelihood analyses. A positive control of *M. graminicola* DNA from the region of Batangas in Philippines was also sequenced and included in the analyses with other *Meloidogyne* spp. sequences retrieved from GenBank. Phylogenetic analyses based on the D2-D3 expansion segment placed *M. salasi* within a large monophyletic group (PP=100, BP=100) composed of several *M. graminicola* isolates and

M. trifoliophila. Similarly, the phylogenetic analysis of the *cox2-16S rRNA* mtDNA region showed a large clade with *M. graminicola* and *M. salasi*. However, a subclade was formed with *M. salasi* and two sequences of *M. graminicola* from Florida and Taiwan (PP=99). A haplotype network analysis (Minimum Spanning Network) showed that these three isolates were the most genetically distant from the main haplotype, which includes isolates from China, Philippines, Bangladesh and India. The inclusion of more DNA markers will help to elucidate this *M. graminicola* complex, which may suggest the presence of cryptic species.

OLD AND NEW ROOT-LESION NEMATODES FROM FLORIDA. **Inserra, R.N.** Division of Plant Industry, DPI-FDACS, Gainesville, Florida 32614-7100, USA.

According to Lehman (2002), 16 root-lesion nematode species occur in Florida on agronomic, ornamental, vegetable crops and fruit trees. The identification of these *Pratylenchus* species was based mainly on morphological analyses. The results of cooperative molecular and morphological studies conducted in the last two decades by taxonomists in the USA and Europe have clarified the taxonomic status of most of these species, which were not identified correctly or confused with other species, some of them new. The identification of a few of the reported species remains still unverified because they have not been found in recent nematode surveys conducted in cultivated and non-cultivated fields, in Florida. At present, only nine species have been identified and characterized morphologically and molecularly using topotype material. These species include: *P. bolivianus*, *P. brachyurus*, *P. coffeae*, *P. floridensis*, *P. hexincisus*, *P. hippeastri*, *P. parafloridensis*, *P. vulnus* and *P. zaeae*. Problems of identification and classification of some *Pratylenchus* species from Florida are presented and discussed using morphological and molecular datasets.

MUESTREO DE NEMATODOS ASOCIADOS AL MAIZ EN LA ZONA CENTRO DE JALISCO. **Ireta, J.¹, N.Y. Zacamo¹, R.G. García¹, and J.F. Pérez¹.** ¹INIFAP C.E. Centro Altos de Jalisco. Jalisco, México.

El maíz es el cultivo más importante en Jalisco, se siembran cerca de 700 mil hectáreas, con un rendimiento medio de 4.0 ton/ha. El rendimiento se ve afectado por una gran cantidad de plagas y enfermedades, las cuales en su conjunto llegan a reducir el rendimiento hasta en un 30-40%. Un factor poco o casi nada estudiado es la presencia y/o daño de los nematodos en maíz (al menos no publicados). En otras regiones como el Estado de México, se ha reportado la presencia del enquistado *Punctodera chalcoensis*, y en Guanajuato se han reportado 14 géneros de nematodos filiformes en maíz, trigo, sorgo, ajo, alfalfa y fresa, sin precisar el género por cultivo; entre los principales están *Pratylenchus*, *Helicotylenchus*, *Psylenchus*, *Tylenchorhynchus* y *Boleodorus*. Se realizó un muestreo exploratorio para conocer la situación actual de los nematodos en el maíz. El muestreo se realizó de enero a febrero del 2013, en 84 localidades distribuidas en 5 regiones agroecológicas. Para la extracción, se utilizó el método “Tamizado-Centrifugado y Gradiente de azúcar”, con una muestra homogénea de 150 g de suelo por cada sitio de muestreo. El conteo de nematodos se realizó bajo un estereoscopio. Las regiones agroecológicas en que se dividió el estudio fueron, Región Ciénega de Chapala, Región Centro, Región Valles, Región Altos y Región Sur, con un valor medio de 14.89, 19.86, 18.75, 20.7 y 147.2 larvas /150 gr de suelo/región respectivamente. En la región Ciénega las localidades con mayor incidencia de nematodos fitófagos fueron Ocotlán y Zapotlán de Rey. En la región Centro, el municipio de Jocotepec resultó con los valores más altos para la presencia de nematodos. En la región Valles, las localidades que sobresalieron fueron San Martín Hidalgo y Acatlán de Juárez, y para la región de Altos, la localidades de Arandas y Tepatitlán fueron las de mayor incidencia de nematodos. La región Ciénega es la más productiva de Jalisco en relación al maíz, sin embargo también es la región más problemática con las plagas del suelo y con la pudrición del tallo causados por el complejo de especies de *Fusarium* sp. En la región Centro, llama la atención el municipio de Jocotepec por las mayores densidades de nematodos; la probable razón de este fenómeno es el que se siembran hortalizas y berries (frambuesa, zarzamora y arándano) en forma intensiva. En la región Valles hay municipios con poca incidencia de nematodos y esto puede deberse a que el maíz entra en rotación con caña de azúcar, y este cultivo dura varios años en el mismo predio y con excesos de agua. La región de los Altos es la más afectada, sin embargo esto está influenciado por los datos de Arandas y Tepatitlán, en estas localidades se usan mucho los abonos orgánicos. Al conjuntar la información por región, se observa una tendencia más estable, es decir que en todas las zonas productoras se tiene la presencia de nematodos. De los 2157 nematodos aislados y asociados al maíz, se identificaron 84.6% como *Pratylenchus* sp., 6.9% *Helicotylenchus* sp, y 8.5% como *Criconemoides* sp., además de un grupo de enquistados que no han sido identificados aún.

REACTION TO *MELOIDOGYNE PARANAENSIS* IN “PIATÃ” COFFEE GENOTYPES. **Ito, D. S.¹, G.H. Sera¹, E. Andreazi², and S.A. Silva¹.** ¹Instituto Agrônômico do Paraná, IAPAR, Londrina, Paraná State, Brazil. ²Universidade Estadual de Londrina, Londrina, Paraná State, Brazil/CAPES.

Coffee is an important crop in Brazil. However, the Brazilian coffee growers currently have a limiting factor caused by the root-knot nematodes, which cause great losses for the coffee crop. The use of coffee cultivars resistant to nematodes is the best choice for managing these nematodes in infested areas because it represents a control method more efficient, economically viable, and environmentally friendly than other control options. Sources of resistance to *Meloidogyne paranaensis* were identified only in *Coffea arabica* and *C. canephora*. The genotype Piatã is a natural F₁ hybrid between *C. dewevrei* and

C. arabica, which has 44 chromosomes. It has been used in backcrosses with the cultivar Catuaí Vermelho (*C. arabica*) in several crop breeding programs in Brazil. Until now, this genotype has not tested for resistance to *M. paranaensis*. The aim of this study was to evaluate the resistance of F₃ genotypes of “Piatã” to *M. paranaensis*. The experiment was conducted at greenhouse in IAPAR agronomical institute, at Londrina, Paraná State, Brazil. The experiments were arranged in completely randomized design with one plant per plot and 10 replications. Five genotypes were evaluated. The cultivar Mundo Novo IAC 376-4 was used such as susceptible check. A set of uninoculated plants was used as a control. The coffee plants were grown in pots containing about 600 ml of substrate (2 of sand: 1 of soil), and were inoculated with 2,000 eggs and juveniles of nematodes when they had four to six pairs of leaves. Assessments for resistance to *M. paranaensis* were performed 150 days after the inoculation. Eggs and second-stage juveniles (J2) were extracted from the root system and the final population density (Pf) was determined as well as the Pf/g of root. The nematode reproduction factor (RF) of each plant was calculated using the formula: $RF = Pf/2,000$. All genotypes had an average RF > 1.0, but there were statistical differences between the genotypes, showing four distinct groups. The RF of susceptible check was 140.5 and of the genotypes T4, T6, T3 and T2 were 2.9, 57.0, 69.7, 79.7, respectively. It is possible that the genotype T4 has quantitative resistance due to the effect of minor genes because the RF was lower than the other genotypes, but above 1. The T4 genotype may also contain qualitative resistance because 50% of plants had an RF < 1. This resistance to *M. paranaensis* may originated from *C. deweyrei* because the other parent of “Piatã” is susceptible. Individual plants of genotype T4 will be selected to advance for next generation aiming to identify resistant homozygous genotypes.

ASSESSMENT OF ILeVO FOR MANAGEMENT OF *MELOIDOGYNE INCOGNITA* IN SOYBEAN. **Jackson, C.S.¹, T.R. Faske², M. Emerson², and K. Hurd²**. ¹University of Arkansas, Department of Plant Pathology, Fayetteville, AR. ²University of Arkansas, Lonoke Research and Extension Center, Lonoke, AR.

Soybean seed treatment with fluopyram (ILeVO) was registered in 2014 to manage soilborne fungi and plant-parasitic nematodes. There is little information on the effectiveness of ILeVO against the root-knot nematode, *Meloidogyne incognita*. Field trials were conducted in 2014 and 2015 to evaluate the field efficacy of ILeVO to manage *M. incognita* on a susceptible and a moderately resistant soybean cultivar. Treatments consisted of ILeVO, Avicta, Poncho/VOTiVO, ILeVO + Poncho/VOTiVO applied as a seed treatment, fluopyram applied as an in-furrow spray, and a non-treated control. The experimental design was a split plot with cultivar as the whole plot and nematicides as sub-plots. Phytotoxicity was observed along the edge of cotyledonary leaves from ILeVO treated seed, but had no impact on soybean stand or vigor. Nematode reproduction in 2015 was lower on Delta Grow DG 4940, a moderately resistant cultivar than the susceptible cultivar, Delta Grow DG 4970. However, the reverse was observed in 2014 between Armor 53-R16 (susceptible) and Armor 55-R22 (moderately resistant). The effect of nematicides on *M. incognita* were similar between years, but differed between cultivars. There was no significant effect on nematode reproduction due to nematicide on the moderately resistant cultivars, but fewer eggs were collected on roots from the susceptible cultivars treated with Avicta, Poncho/VOTiVO, and ILeVO + Poncho/VOTiVO than on the fluopyram in-furrow treatment and non-treated control. Soybean yield was greater in 2014 than 2015; however, there was no interaction between cultivar and nematicide for either year. Overall, a greater yield was observed in both years and across cultivars with ILeVO than the non-treated control. Based on nematode suppression and yield protection, the field performance of ILeVO was similar to other seed treatment nematicides such as Avicta and Poncho/VOTiVO.

VIDEO ANALYSIS SOFTWARE TO MEASURE NEMATODE MOVEMENT WITH APPLICATIONS FOR ACCURATE SCREENING OF NEMATODE CONTROL COMPOUNDS. **Jensen, J.P.¹, Z. Njus², S. Pandey², and G. Tylka¹**. ¹Iowa State University Department of Plant Pathology and Microbiology. 351 Bessey Hall Ames, IA 50011, ²Iowa State University Department of Electrical and Computer Engineering 2215 Coover Hall Ames IA 50011.

Developing nematode control compounds requires the screening of several candidates in a high throughput manner. Operator scoring based on microscopic observation of nematode movement, a simple yes or no, is a common assay used to determine the effects of compounds. However, some nematode species, such as the soybean cyst nematode (SCN), *Heterodera glycines*, may be inactive *in vitro* even when suspended in water. Operator scoring also is prone to human error due to operator fatigue, which can result in false positives and false negatives, and interoperator variability. Therefore, visually scoring nematodes for movement is not an ideal criterion to assess the effects of a compound. To better characterize nematode responses to a given stimulus, we developed video analysis software to calculate the speed and curvature of movement at 13 tracking points along the length of a nematode. The software was used to measure movement of second-stage juveniles (J2s) of SCN exposed to varying concentrations of abamectin, a known nematicide. Newly hatched SCN J2s were incubated in one of five different concentrations of abamectin (0, 0.1, 1, 10, or 100 mg/L dissolved in 1% acetone) for two hours, then washed twice with sterile distilled water. Control treatments included J2s incubated in water or heat-killed J2s. Half of the nematodes were immediately placed on a microscope slide in water with coverslip and subjected to video capture using an upright stereozoom microscope with overhead lighting and dark background. Visual scoring of nematode movement was conducted on the same videos subjected to software analysis. The remaining nematodes were stored at 22 °C in vials of water and used for video capture and visual scoring 24 hours later. Videos consisted of images taken at 63x magnification every 0.1

seconds for 60 seconds. On average, ten J2s were analyzed from each treatment at each time, and the experiment was conducted twice. Visual scoring and software analysis on the same nematodes allowed us to directly compare J2 movement between both assays. In general, the visual scoring method resulted in variable results amongst different operators for J2s from most treatments. In contrast, both measurements of movement (speed and change in curvature) in the software analysis produced consistent and similar results amongst all treatments levels. Furthermore, the software was able to detect significant differences in average speed and average change in curvature of the nematodes among the concentrations of abamectin and among the tracking locations along the J2's body. There were no differences ($P > 0.05$) in results between the data collected at 0 and 24 hours. Data obtained from visual scoring is limited to one parameter, movement, whereas the software enables us to detect minute differences in speed and curvature of nematode movement that might be missed with visual scoring. By tracking multiple parameters with the software, we can develop a more complex understanding of how compounds may affect nematodes, which could give insight into sub-lethal effects and help determine the effective concentration compounds for nematode control.

POTENTIAL NEW NEMATODE MANAGEMENT AGENTS FOR TOBACCO PRODUCTION IN VIRGINIA. Johnson, C. Southern Piedmont Agricultural Research and Extension Center, 2375 Darvills Road, Blackstone, VA, 23824, and Dept. of Plant Pathology, Physiology, and Weed Science, Virginia Tech, Blacksburg, VA 24061.

Flue-cured tobacco remains an important agricultural crop in Virginia. Crop rotation is extensively practiced by growers, and resistant cultivars are commonly available and frequently planted, but many fields still require treatment for control of a tobacco cyst nematode (TCN - *Globodera tabacum solanacearum*). Because only a few soil fumigants remain available to growers for TCN control, a TCN nematicide trial is conducted annually to identify possible new "nematode management agents" to limit TCN reproduction and increase tobacco yield, while at the same time increase environmental sustainability and applicator safety. All trials were conducted in pre-selected, naturally-infested fields at the Southern Piedmont Center to maximize the size and uniformity of initial TCN populations, and were arranged each year in a randomized complete block design with 4-6 replications. TCN nematode population densities were estimated from soil samples collected before treatment application, mid-season, and after final crop harvest. Two plant samples were also collected from each plot mid-season to enumerate TCN juveniles in 1g subsamples from feeder roots. Leaves were harvested from each plot sequentially as they ripened in order to estimate cured leaf yield. Comparisons of five rates of MCW2 (Nimitz - fluensulfone, ranging from 4.2 to 17.3 L/ha) to an untreated control, Vydate (oxamyl), and 10 gal Telone II (1,3 dichloropropene) found no statistically significant differences in TCN/g of feeder root and leaf number/plant in mid-season, or yield in 2010 and 2011. Single or multiple applications of 16.8, 22.4, or 33.6 kg Ditera/ha didn't reduce TCN/g root or increase plant growth or yield in 2010-2012. Various Neem oil application strategies failed to reduce TCN in roots or increase plant growth or yield in 2013. Apparent reductions in TCN/g of feeder root after 2-3 foliar applications of Movento (spirotetramat) weren't statistically significant, but increased leaf number and yield were similar to the nematicide standards in 2012 and intermediate to those from Vydate and Telone in 2013. Preplant incorporation of 280-1,345 kg Mustgro (mustard seed meal, generating allyl isothiocyanate [AITC])/ha was not associated with statistically significant reductions in TCN/g root or increased plant growth or yield in 2011, but the 2,242 kg/ha rate increased mid-season leaf numbers and percent flowering in 2012, similarly to Telone. Fumigation with 281 or 374 L Dominus (AITC)/ha resulted in intermediate gains in topping and yield versus the untreated control and Telone in 2014, and similar results were observed for the 281 L/ha rate in 2015. Fumigation with 281 L IRF266 (AITC + chloropicrin)/ha reduced TCN/g root and increased leaf numbers in mid-season, reduced days to flowering, and increased yield in 2015. Products containing fluopyram (Velum Total, Luna Privilege) reduced TCN/g root in 2014 and 2015 and increased yield in 2015, but not in 2014.

PROMISING APPROACH FOR IMPROVING THE EFFECT OF SILVER NANOPARTICLE APPLICATIONS IN SOIL FOR MELOIDOGYNE INCOGNITA MANAGEMENT: SYNTHESIS AND APPLICATION. Kalaiselvi, D.¹, M. Rajanandhini¹, P. Sundararaj¹ S.L. Hafez² and N. Sundararaj³. ¹Bharatiar University, Coimbatore-641046, Tamilnadu, India. ²U of I Parma REC, 29603 U of I Lane, Parma, ID 83660, USA. ⁴Kumaraguru College of Technology, Coimbatore, India.

Silver nanoparticles (AgNPs) are biologically active ingredients against a wide range of insects, microbes and nematodes. Several plant extracts are used as reducing agents in the nanoparticle synthesis process, thereby it is possible to reduce environmental pollution. In the present study an attempt was made using the plant extract of *Euphorbia tirucalli* as a reducing agent for the synthesis of AgNPs. Characterization studies such as UV-vis spectrophotometer, Scanning Electron Microscope (SEM), X-ray diffraction (XRD) and Fourier transform infrared (FTIR) confirmed the physical and chemical properties of AgNPs. Nematicidal activity of synthesized nanoparticles were evaluated by direct exposure of *Meloidogyne incognita* second stage juveniles (J2), hatching inhibition and infectivity studies at different concentrations ranging from 0.1 to 1 µg/ml. It was observed that direct exposure of J2 to AgNPs at the concentration of 1 µg/ml resulted in 99% mortality within a 1 hr period as well as 100% inhibition of egg hatching. AgNPs also significantly reduced the infectivity of *M. incognita* on tomato seedlings.

NEW SOYBEAN CYST NEMATODE, HETERODERA SOJAE N. SP. (NEMATODA: HETERODERIDAE) FROM KOREA. **Kang, H.¹, G. Eun¹, N.S. Park², D.G. Kim², and I.S. Choi¹.** ¹Plant Bioscience Dept., Pusan National University, Miryang, 50463, KOREA, ²Nematode Research Center, Life and Industry Convergence Research Inst., Pusan National University, Miryang, 50463, KOREA.

New soybean cyst nematode, *Heterodera sojae* n. sp. was found from root of soybean in Korea. Cysts of *H. sojae* n. sp. appeared more round, shining and darker than that of *H. glycines*. Morphologically, *H. sojae* n. sp. differed from *H. glycines* by fenestra length (23.5-54.2 μm vs 30-70 μm), vulval silt length (9.0-24.4 μm vs 43-60 μm), tail length of second-stage juvenile (J2) (54.3-74.8 μm vs 40-61 μm) and hyaline part of J2 (32.6-46.3 μm vs 20-30 μm). It is distinguished from *H. elachista* by larger cyst (513.4-778.3 μm \times 343.4-567.1 μm vs 350-560 μm \times 250-450 μm), longer stylet length of J2 (23.8-25.3 μm vs 17-19 μm). In molecular analysis of rRNA, LSU D2-D3 segments and ITS gene sequence show that *H. sojae* n. sp. is more close to rice cyst nematode *H. elachista* than *H. glycines*. *H. sojae* n. sp. is not rare in soybean fields in Korea.

TYLENCHID ENTOMOPARASITES ISOLATED FROM TWO LONGHORN BEETLE SPECIES BELONGING TO THE SUBFAMILY SPONDYLINAE. **Kanzaki, N.¹, R.M. Giblin-Davis², R. Gonzalez², Y. Trujillo², and A.E. Hajek³.** ¹Forestry and Forest Products Research Institute, ¹Matsunosato, Tsukuba, Ibaraki 305-8687 Japan, ²Fort Lauderdale Research and Education Center, University of Florida/IFAS, 3205 College Avenue, Davie, FL 33314-7799, USA, ³Cornell University, Dept. of Entomology, 6126 Comstock Hall Ithaca NY 14853-2601.

Entomoparasitic nematodes belonging to the infraorder Tylenchomorpha typically have multiple adult morphs, e.g., parasitic female, infective female, and free-living (mostly mycophagous or non-feeding) adult male and female morphs. Some nematode species in this category are considered as potential biocontrol agents of their respective insect hosts. However, partially because of their complex life histories with multiple forms and morphological similarity among superfamilies, their systematics are not fully understood. In the present study, two tylenchid entomoparasites were discovered and isolated from two Spondylinae longhorn beetles, *Spondylis buprestoides* from Japan and *Asemum striatum* from New York, U.S.A. The parasitic females isolated from *S. buprestoides* have long (ca. 5-6 mm) bodies, a clearly observed stylet retracted into the body and a degenerate anus and rectum, similar to some neotylenchids and allantonematids. Discordantly, the nematodes isolated from *A. striatum* were all parasitic juveniles with no clear generic/family-specific morphological character(s) observed. Three molecular barcoding loci, i.e., near-full-length small subunit (SSU), D2-D3 expansion segments of the large subunit (D2-D3 LSU) ribosomal RNA genes, and partial mitochondrial cytochrome oxidase subunit I (mtCOI), were sequenced from the two different host-associated tylenchid entomoparasites and compared with sequences deposited in the GenBank database. The BLAST search suggested the closeness of these two species with *Deladenus* spp., which was corroborated by phylogenetic analysis with the SSU sequences. These two species were closely-related to each other, i.e., forming a well-supported clade basal to and monophyletic with the *siricidicola* clade of *Deladenus*. The inferred phylogenetic closeness and biological similarity of these two species, i.e., they both parasitize the body cavity of Spondylinae beetles, may suggest the presence of a Spondylinae-associated lineage. Based on their phylogenetic status, they may belong to the genus *Deladenus*. However, the parasitic female of the *S. buprestoides*-associate has some minor typological differences with *Deladenus*, e.g., the *S. buprestoides*-associate has a totally degenerate anus and rectum, whereas in current members of *Deladenus* these features can be seen. More detailed morphological observations based on further isolates and cultured specimens are necessary to identify and describe those two newly-discovered nematode species.

ASSESSMENT OF GEOGRAPHIC ISOLATES OF ENDEMIC POPULATIONS OF *ROTYLENCHULUS RENIFORMIS* AGAINST SELECTED COTTON GERMPLASM LINES. **Khanal, C., E.C. McGawley, and C. Overstreet.** Department of Plant Pathology and Crop Physiology, Louisiana State University, Baton Rouge, LA 70803.

Previous reports indicate presence of variation in reproduction of geographic isolates of reniform nematode (*Rotylenchulus reniformis* Linford and Oliveira). Lack of reniform resistant cotton cultivars and existence of the reproductive variability in reniform nematodes add challenges in reniform nematode management. A greenhouse study was conducted to assess reproduction of four geographic isolates (from Ouachita, Rapides, East Carroll, and Tensas parishes) of reniform nematode (*Rotylenchulus reniformis* Linford and Oliveira) on five upland cotton (*Gossypium hirsutum* L.) germplasm lines (TX 110, MT2468 REN1, MT2468 REN3, M713 REN1, M713 REN5) and Stoneville LA 887. Thirty-four days after inoculation, vermiform life-stages of the nematode in soil and egg masses present on root systems were enumerated. Data were examined by analysis of variance. LA Stoneville 887, MT2468 REN3 and M713 REN5 showed significant variation in numbers of vermiform stages in soil and numbers of egg masses per gram of root. Greatest reproduction of reniform nematodes was observed on LA 887 while lowest reproduction was observed on M713 REN5. TX 110 and M713 REN5 were statistically similar in terms of vermiform stages and egg masses per gram of root. Among four reniform nematode isolates, East Carroll had lowest reproduction compared to other isolates across the six cotton genotypes. Results suggest existence of virulence phenotypes among endemic populations of reniform nematodes in Louisiana. Results from this study would be useful for cotton breeders involved in development of resistant cotton cultivars and management decisions by producers.

EFFECT OF LEAF MEAL SOIL AMENDMENTS OF NON-CROP PLANT SPECIES USED IN TRADITIONAL MEDICINE IN SOUTH AFRICA ON *MELOIDOGYNE INCOGNITA* REPRODUCTION AND YIELD OF TOMATO. **Mbokota C.K.¹, A.H. Mc Donald^{2,†}, M.S. Daneel¹, and D. DeWaele^{2,3}**. ¹Agricultural Research Council-Institute of Tropical and Subtropical Crops, Private Bag X11208, Nelspruit 1200, South Africa, ²Unit for Environmental Sciences and Management, North-West University, Private Bag X6001, Potchefstroom 2520, South Africa, ³Laboratory for Tropical Crop Improvement, Department of Biosystems, Faculty of Bioscience Engineering, University of Leuven (KU Leuven), Willem de Croylaan 42, 3001 Heverlee, Belgium. † A.H. McDonald passed away on October 8th 2014.

Crop failure as a result of nematode infection is frequently reported from resource-poor farming areas and is a major constraint for household food security in South Africa. Alternative low-input, cost-effective and environmentally-friendly nematode management strategies need to be developed to provide disadvantaged rural people with techniques to regain and maintain acceptable levels of food production. Powdered leaf meal of five non-crop plant species used in traditional medicine in South Africa were examined for their efficacy as a soil amendment in controlling *Meloidogyne incognita* race 2 on tomato in glasshouse, microplot and field trials. A randomized complete block design layout was used with 18 treatments, replicated 6 times. Each plant was inoculated with \pm 3,000 *M. incognita* race 2 eggs and second-stage juveniles (J2). Our results indicate that nematode eggs and J2 numbers were significantly lower in tomato plants treated with the powdered leaf meals compared to the untreated control plants. The comparative superior efficacy of *Cissus cactiformis*, *Maerua angolensis* and *Tabernaemontana elegans* and their comparable superior performance to the nematicide fenamiphos provide further evidence of the potential usefulness of these powdered leaf materials in the management of plant-parasitic nematodes.

EXOSOME-LIKE VESICLES: A MECHANISM FOR EFFECTOR SECRETION AND HOST MANIPULATION IN ANIMAL PARASITIC NEMATODE INFECTION. **Kimber, M.J.¹, H. Harischandra¹, M. Zamanian², L.M. Fraser¹ and L.C. Bartholomay³**. ¹Department of Biomedical Sciences, Iowa State University, Ames, IA 50011; ²Department of Molecular Biosciences, Northwestern University, Evanston, IL 60208; ³Department of Pathobiological Sciences, University of Wisconsin-Madison, Madison, WI 53706.

Parasitic nematodes of medical and veterinary importance continue to be global health concerns, posing complex challenges for effective disease treatment and control. A major obstacle to the development of new control strategies is our poor understanding of parasitic nematode biology. For example, the interaction between parasite and host during infection, development and persistence of nematode disease is dynamic and delicately balanced. Manipulation of this interface to the detriment of the parasite is a novel approach to disease therapy but is prevented by our very limited understanding of the host-parasite relationship. Here we describe a novel mechanism by which filarial nematodes including *Brugia malayi* modulate host biology to benefit the parasite, through small, regulatory RNAs and proteins delivered via specific extracellular vesicles called exosomes. We used electron microscopy and nanoparticle tracking analysis to show that all life cycle stages of *B. malayi*, an etiological agent of Lymphatic Filariasis, secrete prodigious quantities of exosome-like vesicles (ELVs). Proteomic profiling revealed that these ELVs contain putative effector proteins that are known to be necessary for development within the host as well as proteins associated with RNA-binding. Correspondingly, RNA-seq analysis of ELV cargo revealed a complex and diverse small RNA complement dominated by microRNAs (miRNA). Significantly, a cohort of these miRNAs have homology to host miRNA and host miRNA seed sequences, suggesting a potential mechanism by which parasites might affect host gene expression. Use of lipophilic dyes allowed visualization of ELV internalization by host macrophages, key mediators of the early immune response to infection. Interestingly, parasite ELV internalization elicited unexpected activation phenotypes in host macrophages in a parasite life stage-specific manner. These data support a working hypothesis that human and animal parasitic nematodes secrete ELVs that contain effector molecules capable of host manipulation at the cellular and transcriptional levels to create conditions favorable to parasitism.

EVALUATION OF STEAM FOR *MELOIDOGYNE ARENARIA* CONTROL IN PRODUCTION OF IN-GROUND FLORICULTURE CROPS IN FLORIDA. **Kokalis-Burelle, N.¹, D.M. Butler², J. Holzinger³, and E.N. Roskopf¹**. ¹USDA-ARS, U.S. Horticultural Research Lab, Ft. Pierce, FL 34945, ²University of Tennessee, Knoxville, TN, ³Holzinger Flowers, Inc., Palm City, FL.

Steam and soil solarization were investigated for control of the root-knot nematode *Meloidogyne arenaria* in two years of field trials on a commercial flower farm in Florida. The objective was to determine if pre-plant steam treatments in combination with solarization, or solarization alone effectively controlled nematodes compared to methyl bromide (MeBr). Trials were conducted in a field with naturally-occurring populations of *M. arenaria*. Treatments were; solarization alone, steam treatment after solarization using standard 7.6 cm-diameter perforated plastic drain tile (steam 1), steam treatment following solarization using custom-drilled plastic drain tile with 1.6 mm holes spaced every 3.8 cm (steam 2), and MeBr applied at 392 kg/ha 80:20 MeBr:chlorpicrin. Drain tiles were buried approximately 25 cm deep with four tiles per 1.8 m by 30 m plot. Steam application followed the four week solarization period in mid-October. All steam was generated using the Sioux propane boiler system. Plots were steamed for sufficient time to reach the target temperature of 70 °C for 20 min. Solarization plastic was retained on the plots during steaming and plots were covered with a single layer of carpet padding to

provide additional insulation. The floriculture crops larkspur (*Delphinium elatum* and *Delphinium x belladonna*), snapdragon (*Antirrhinum majus*), and sunflower (*Helianthus annuus*) were produced according to standard commercial practices. One month after treatment in both years of the study, soil populations of *M. arenaria* were lower in both steam treatments and in MeBr compared to solarization alone. At the end of the season in both years, galling on *Delphinium elatum*, snapdragon, and sunflowers was lower in both steam treatments. Both steam treatments also provided control of *M. arenaria* in soil at the end of the season comparable to, or exceeding that of MeBr. Both steam treatments also reduced *M. arenaria* in snapdragon roots comparable to, or exceeding control with MeBr. *M. arenaria* in soil increased in solarization alone following treatment. Solarization alone also had higher gall ratings on *Delphinium elatum*, snapdragon, and sunflower than all other treatments. Although steam provided excellent control of *M. arenaria* in this study, plant growth was reduced, indicating a possible deleterious effect of steam on beneficial soil microorganisms. Additional research on identifying microorganisms important to maintaining optimum plant growth and on supplementing or enhancing natural populations of beneficial soil microorganisms to improve plant growth following steam and other soil disinfestation treatments is currently underway.

DOMINUS® FOR MELOIDOGYNE ARENARIA AND WEED CONTROL IN FLORIDA CUT FLOWER PRODUCTION. Kokalis-Burelle, N.¹, J.C. Hong¹, T.N. Ivy², J. Holzinger³, and E.N. Roskopf¹. ¹USDA-ARS, U.S. Horticultural Research Lab, Ft. Pierce, FL 34945; ²Isagro USA, Inc, Morrisville, NC 27560; ³Holzinger Flowers, Inc., Palm City, FL 34991.

Two years of field research was conducted at a commercial flower farm in Florida to evaluate DOMINUS® (allylthiocyanate (AITC); 374 L/ha) for nematode and weed control compared to methyl bromide (MeBr 392 kg/ha 80:20 MeBr: chlorpicrin). Dominus is a biofumigant registered for conventional and organic farms. The field site had high populations of *M. arenaria* and weeds including nutsedge (*Cyperus rotundus*), Carolina geranium (*Geranium carolinianum*), and goosegrass (*Eleusine indica*). Floral crops grown were sunflower, millet, zinnia, celosia, and tuber rose. MeBr and Dominus were shank applied to 3.6 m x 33.5 m plots and covered with totally impermeable film (TIF) for 2 weeks. Plots were replicated 4 times and split among cut flower species. Soil samples were collected throughout the season for nematode analysis. Weed emergence was evaluated in two 1-m² areas within each plot. After harvest, plant growth and disease were assessed, and nematodes were isolated from soil and roots. In the first season, low numbers of *M. arenaria* juveniles (J₂) were present in pre-treatment soil samples and no *M. arenaria* J₂ were detected in either treatment 18 days after application. There were no differences among genera of plant-parasitic or non-parasitic nematodes between treatments with numbers of plant parasites averaging fewer than 10 J₂/g of soil. At snapdragon harvest, there were no differences between root condition ratings and root galling. Roots harvested from Dominus plots were heavier than roots from MeBr. At larkspur and delphinium harvest there were no differences between treatments, except that root galling in MeBr was slightly higher in delphinium. Lupin had larger root systems and stems from Dominus plots. No differences occurred in *M. arenaria* in roots or soil of snapdragons, larkspur, delphiniums, and lupin. Total number of marketable cut stems harvested from Dominus and MeBr plots was not different for any of the flower species tested. Weed control was similar between treatments and neither provided control of Carolina geranium (*Geranium carolinianum*). In the second season with plots in the same location, two highly susceptible crops, *Celosia argentea* (cockscomb) and *Helianthus annuus* (sunflower) were grown. The only weed species present immediately after treatment was annual sedge (*Cyperus compressus*), which occurred only in the Dominus treatment. *M. arenaria* in soil were not different between treatments, but were numerically higher in MeBr compared to Dominus. Celosia root populations were similar between treatments and roots were equivalently galled. Plants from Dominus-treated plots were taller than those in MeBr. *M. arenaria* did not increase at the end of the season with no differences between treatments for soil or root nematodes. Overall, there were no differences in the total number of marketable stems from either celosia or sunflower plots. Dominus was comparable to MeBr for control of nematodes and weeds in cut flower production in Florida.

DIVERSITY AND BIOCONTROL POTENTIAL OF FUNGI ISOLATED FROM CYST NEMATODE *GLOBODERA PALLIDA*. Kooliyottil, R.¹, L.M. Dandurand¹ and G.R. Knudsen². ¹ Plant, Soil, and Entomological Sciences Department, ² Soil and Land Resources Division, University of Idaho, Moscow, ID, 83844.

The potato cyst nematode, *Globodera pallida*, is one of the most important pests of potato worldwide. Due to regulatory considerations and potential environmental impacts, control options for this nematode are becoming increasingly limited. Biological control agents are potentially valuable tools because of their ability to parasitize eggs, and induce a systemic resistance in plants. To determine the diversity of potential biocontrol fungi, pathogenic fungi were isolated from individual eggs collected from cysts found in infested fields. Seven different fungi were isolated by plating eggs onto potato dextrose agar (PDA) containing streptomycin (50 ug/ml), and identified using molecular and morphological taxonomic approaches. The fungal species were identified as *Chaetomium globosum*, *F. oxysporum*, *F. solani*, *Fusarium tricinctum*, *Microdochium bolleyi*, *Purpureocillium lilacinus*, and *Plectosphaerella cucumerina*. All seven isolates were tested under greenhouse conditions to assess their efficacy as biological control agents. *Solanum tuberosum* was grown in micro-rhizosphere chambers (ROC) and conidial suspension of the test fungi were inoculated at a rate of 10⁷ conidia/ml. Seven days after inoculation, hatched second stage juveniles (J₂) were inoculated (200 J₂s/plant). Four days post inoculation, roots were sampled and stained with acid fuchsin. Infection levels in each sample were assessed. Among the 7 fungi tested, *C. globosum*,

F. solani, *F. oxysporum* and *P. lilacinus* showed 74.01%, 63.27%, 62.73% and 40.16% reduction, respectively, in infection by *G. pallida* in colonized roots compared to non-colonized roots. *P. cucumerina*, *M. bolleyi*, and *F. tricinctum* did not significantly reduce infection of *G. pallida* compared to the control. Further studies are being conducted to determine whether these fungi can directly infect *G. pallida* eggs.

TRANSCRIPTOME OF *SOLANUM SISYMBRIIFOLIUM* AND *SOLANUM TUBEROSUM* FROM *GLOBODERA PALLIDA* INFECTED ROOT CELL. **Kooliyottil, R., L. M. Dandurand, J. Kuhl, A. Caplan, and F. Xiao.** Plant, Soil, and Entomological Sciences Department, University of Idaho, Moscow, ID, 83844.

Solanum sisymbriifolium Lam stimulates hatch of *Globodera pallida* (Stone) Behrens (pale cyst nematode, PCN), but does not allow nematode reproduction. A non-destructive imaging tool, coupled with the fluorescent stain PKH26 was used to investigate the behavior of *G. pallida* in susceptible *Solanum tuberosum* L and resistant *S. sisymbriifolium*. We observed a resistant reaction in *S. sisymbriifolium* 24-48 hours post infection. However, infection by *G. pallida* was much lower in *S. sisymbriifolium* than in *S. tuberosum*, and development of *G. pallida* was arrested in *S. sisymbriifolium* roots. Dead nematodes were also found in *S. sisymbriifolium* roots at 4 days post infestation (dpi). Microaspiration of individual cells allows for a minimally invasive method of sampling, instead of sampling whole root. *S. tuberosum* and *S. sisymbriifolium* were grown in micro-rhizosphere chambers (ROC; Advance Science Tools, USA) under greenhouse conditions and PKH26 stained *G. pallida* were inoculated at the root tip. After 24 hours the infected plant root cells were microaspirated by using a micro capillary and micro manipulator (Celltram vario, Eppendorf, USA) attached with the fluorescent microscope (Leica, DMI 3000B, Germany). Total RNA was isolated from the aspirated samples by using an Agencourt RNAdvance Tissue total RNA extraction Kit (Beckman Coulter, USA). The purity of the RNA was determined by using a fragment analyzer (Advanced Analytical Technologies Inc., USA) and RNA Quality Number (RQN) was found to be 7.8. Preparation of cDNA was performed according to the procedure described in the SUPERSRIPT® II reverse transcriptase (Invitrogen) using oligo (dT) primer and dNTP mix. Further verification was done by amplification of representative housekeeping genes. Our study shows that PKH26 staining coupled with micro-ROC enables microaspiration of *G. pallida* infected *S. tuberosum* and *S. sisymbriifolium* root cells for transcriptome studies. Further studies to understand the molecular mechanisms of non-host infection processes are underway.

EFFICACY OF REDUCED-IMPACT NEMATICIDES FOR MANAGEMENT OF DAGGER NEMATODE IN PEACH. **Kotcon, J.** Division of Plant and Soil Sciences, West Virginia University, P.O. Box 6108, Morgantown, WV 26506.

Peach Stem Pitting, caused by Tomato RingSpot Virus and transmitted by Dagger nematode, is the most important nematode problem in West Virginia peach orchards, however, few chemical nematicides are available for use on bearing trees. Two trials were established in existing peach orchards, one at the Kearneysville Tree Fruit Research and Education Center in Jefferson County, WV and the other in a commercial orchard in Hampshire County, WV. Three replicates of each of the following treatments were established on May 8, 2014, with treatments repeated May 13, 2015: 1) Untreated control, 2) Ditera DF (100 lb/A), 3) Ecozin-Sp (Spring applications only, 30 oz/A repeated at 14-day intervals), 4) Ecozin-Sp&F (Spring and Fall applications, Fall applications were on Oct. 7 and Nov. 4, 2014. No materials were applied in Fall 2015), 5) Movento foliar spray (9 oz/A applied with air blast sprayer), and 6) Nema-Q (3 Gal/A). Each product was applied at the maximum label rate as either a soil-applied spray, or foliar spray as per label recommendations. Soil-applied materials were incorporated via sprinkler irrigation and natural rainfall. Tree response to treatment was determined by measuring trunk diameter, and incidence of Peach Stem Pitting. Trunk diameter measurements and nematode sampling were taken on May 8, June 19 and Oct. 7, 2014, and on May 13, July 28, and Nov. 3, 2015. Peach yield data were collected by harvesting all fruits on the center tree in each plot on Aug. 3, 2015. *Xiphinema rivesi* was the dominant dagger nematode at the Kearneysville site whereas the Hampshire County site was dominated by *Xiphinema americanum*. Other plant parasitic nematodes observed included Spiral nematode (*Helicotylenchus*) and low levels of Lesion nematode (*Pratylenchus*) and Ring nematode (*Mesocriconemella*). Predatory nematodes (*Mononchidae*) were also common. None of the treatments exhibited nematode population densities that differed significantly from untreated controls, nor did tree growth, peach yield or survival differ significantly among nematicide treatments. The Hampshire County orchard continued to exhibit significant tree mortality and symptoms typical of Peach Stem Pitting, however, it is likely that most of the affected trees were already infected when the experiment began. Inability to adequately incorporate these products in established orchards may limit their potential.

IMPACT OF FUMIGATION ON SOYBEAN VARIETIES AGAINST *ROTYLENCHULUS RENIFORMIS*. **Kularathna, M.¹, C. Overstreet¹, E.C. McGawley¹, D.M. Xavier¹ and F.M.C. Godoy¹.** ¹LSU Agricultural Center, Department of Plant Pathology and Crop Physiology, 302 Life Sciences Building, Baton Rouge, LA 70803.

The reniform nematode (*Rotylenchulus reniformis*) is one of the major pests on both soybean and cotton in the southern USA. Yield losses from this nematode to soybean have been reported to be greater than from cotton. Resistance in soybean cultivars against reniform nematode is not consistent with different geographical isolates of the pathogen. Resistant soybean cultivars recommended for Louisiana are based on the evaluations done in Arkansas with their native reniform populations.

Field experiments were conducted during 2014-2015 in Tensas parish Louisiana, to evaluate the response of nematicide on 18 soybean cultivars against endemic *R. reniformis* populations. These soybean cultivars were categorized as susceptible, moderately resistant and resistant, according to studies conducted in Arkansas. In 2014 trial, fumigated (1,3-dichloropropene) and non-fumigated treatments representing 9 cultivars were employed. Nematode population data were collected at-planting, midseason and at-harvest. Application of the nematicide significantly reduced nematode population levels at all three sampling intervals. Out of the 9 cultivars, Delta Grow 4940 and S11-20354 had the lowest reniform nematode populations without fumigation at midseason and at-harvest. The cultivar R04-1268 produced the highest nematode populations in the absence of fumigation at both midseason and at-harvest. In both the presence and absence of the fumigant, lowest yields were observed with R04-1268, MPG 4714N, and Asgrow 4534. In 2015, another 9 soybean cultivars were tested in the same field with and without the fumigant. Similar to 2014, nematode population data were collected at-planting, midseason and at-harvest. Application of the nematicide significantly reduced nematode population levels at all three sampling intervals following a similar trend from the previous year. In this study, Asgrow 5535, Dyna Grow s52RY75 and Delta Grow 5230 had the lowest reniform nematode populations without fumigation at-harvest. The cultivar Delta Grow 5625 produced the highest nematode populations at harvest in nematicide untreated plots and resulted in significantly lower yield. These studies indicated that even with a different reniform isolate, soybean cultivars considered as resistant were able to hold their resistant against a population of reniform nematode in Louisiana.

EVIDENCE FOR SUPPRESSION OF *MELOIDOGYNE HAPLA* BY *PASTEURIA* IN CONNECTICUT. **LaMondia, J.A.** The Connecticut Agricultural Experiment Station Valley Laboratory, P.O. Box 248, Windsor, CT 06095.

Populations of the northern root-knot nematode *Meloidogyne hapla* declined over time in experimental field microplots infested in 1995 and repeatedly inoculated with the nematode. The potential cause of the nematode decline was investigated. *Pasteuria* bacteria endospores were observed on the cuticles of up to 60% of *M. hapla* juveniles recovered from soil in these microplots. Soil was sampled from all microplots, bulked, dried and lots of 3 kg were each either left as non-treated, microwaved for 4.75 minutes or autoclaved for 1 hour each on two successive days. Five replicate pots each containing 500 cm³ of these three soil treatments were inoculated with 2,500 eggs and juveniles of *M. hapla* in 3 ml water. After 5 weeks, plants grown in autoclaved soil had more galls (465) than those grown in untreated soil (203); gall numbers from microwaved soil were intermediate (267) ($P=0.04$). In another study, hatched juveniles were added to flasks containing 25 cm³ of soil in 50 ml water and shaken for 7 days. Nematodes were recovered by sugar centrifugation and the number of endospore-free or endospore-encumbered juveniles counted. Autoclaved soil had 8 of 20 juveniles with endospores; microwaved soil had 10 of 20 juveniles with endospores, and non-treated soil had 15 of 20 juveniles with attached endospores. *Meloidogyne hapla* juveniles that were exposed to non-treated soil were added to pots with tomato transplants. After 6 weeks, *Meloidogyne* females that developed within galls were dissected out of roots and examined. Endospore-filled females were observed, indicating that this isolate of *Pasteuria* infected juveniles and produced endospores in *M. hapla*. The association between *Pasteuria* and *M. hapla* appears to contribute to suppression of the nematode in Connecticut soils.

COPULATORY PLUGS, MALE-MALE COMPETITION AND FECUNDITY IN MOSQUITO-PARASITIC NEMATODE *STRELKOVIMERMIS SPICULATUS*. **Lan, Y.-H., Y. Wang, and R. Gaugler.** Center for Vector Biology, Rutgers University.

The mosquito-parasitic nematode, *Strelkovimermis spiculatus* (Mermithidae: Nematoda) emerges from hosts and aggregates to form mating clusters characterized by intense male-male competition for females. Successful males deposit a copulatory plug over the female vulva after mating. In choice experiments, males strongly preferred virgin females, whereas plugged females were ignored. Males were not observed attempting to remove the plug or attempting to mate. Females with a copulatory plug repelled males. The observed chemical repellency was independent of females since excised plugs showed the same negative male response. The plug contributes significantly to female fitness since our removal of the plug after mating reduced female fecundity by 92.1%. The mechanism for these results is unclear. Our initial hypothesis that the plug provides a nutritional gift was rejected since there was no post-mating reduction in plug size that would have indicated absorption. We are now testing our default hypothesis that the plug blocks sperm leakage.

PLANT-PARASITIC NEMATODES ASSOCIATED WITH BREADFRUIT IN HAWAII. **Lau, J.-W., B.S. Sipes¹, S. Marahatta², K.-H. Wang¹, and D. Ragone³.** ¹University of Hawai'i at Mānoa, Honolulu, 98622, ²Kaua'i Community College, Lihue, 96766, and ³National Tropical Botanical Garden, Kalaheo, 96741.

Breadfruit (*Artocarpus altilis*) is a high yielding, low input starch crop of tropical and subtropical areas. Transported in voyaging canoes with the first inhabitants of the Hawaiian Islands, the breadfruit tree is being revisited and embraced as a food crop and landscaping ornamental. Breadfruit has been traditionally cultivated from root cuttings, however development of tissue culture techniques allows for mass cloning and commercial distribution of select varieties. Global concerns over food security with a focus on traditional underutilized crops coupled with modern propagation techniques have increased cultivation of breadfruit trees as an orchard monocrop in Hawaii and elsewhere. Plant-parasitic nematodes (PPNs) are

obligate microscopic roundworms that feed on plants and cause significant production losses to crops around the world. Although Hawai'i's isolation and quarantine regulations reduce the threat of plant pathogen introduction, soil-borne pathogens like PPNs can travel undetected in imported plants, infested growing media, and roots imported for propagation of breadfruit. Research on PPNs on breadfruit in Hawai'i is unavailable. Consequently, a survey of PPNs associated with the roots and rhizosphere of breadfruit in Hawai'i was conducted on the islands of Kaua'i, Maui, and O'ahu. Roots and soil were collected from residences, commercial properties, breadfruit orchards, or botanical gardens and assayed for nematodes. Nematodes were extracted from soil by rolling and sieving followed by centrifugal floatation, whereas those from roots were extracted by placing roots in a mist chamber. *Helicotylenchus*, a Heteroderid spp., *Meloidogyne*, *Mesocriconema*, *Paratylenchus*, *Pratylenchus*, *Rotylenchulus*, *Scutellonema*, and *Tylenchorhynchus* spp. were found during the survey. *Helicotylenchus* was the most widespread species found on all islands surveyed, occurring in 75% of the samples on Kaua'i. On Maui, *Rotylenchulus* and *Pratylenchus* were most common, occurring in 70% of the samples assayed. On O'ahu, *Pratylenchus* was the most prevalent PPN, found in 85% of the samples. A Heteroderid was found in samples from botanical gardens on each island surveyed. The feeding behaviors of *Meloidogyne*, *Pratylenchus*, and *Rotylenchulus* make these PPNs a greater threat to breadfruit than the other PPNs found. A pathogenicity test of these three nematode species on breadfruit will be conducted. The presence of PPNs on breadfruit could indicate potential disease and crop damage.

STUDY ON HOST-SEEKING BEHAVIOR AND CHEMOTAXIS OF ENTOMOPATHOGENIC NEMATODES WITH PLURONIC GEL. Li, C.¹, J. You¹, Y. Hu¹, X. Zhou², Y. Yu², and C. Wang¹. ¹Key Laboratory of Mollisols Agroecology, Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences, Harbin 150081, China, ²Shandong Key Laboratory of Plant Virology, Institute of Plant Protection, Shandong Academy of Agricultural Sciences, Jinan 250100, China.

Pluronic F-127 gel (PF127) has been proved to be a powerful medium to study host-finding behavior and chemotaxis for plant-parasitic nematodes. Our previous study demonstrated pluronic gel could also be utilized to study host-habitat seeking behavior of entomopathogenic nematode (EPN), natural enemy of root-feeding insect pests. In this study, PF127 was used to study EPN host finding behavior and determine EPN attraction to acetic acid (pH gradient). The root gnat (*Bradysia odoriphaga*), a damaging pest on Chinese chive in China, was immersed into the pluronic gel mixed with EPNs in a petri dish. Once the gel solidified, the sticky gel immobilized the root gnat. We observed that some EPN species quickly moved toward the root gnat within a few minutes after the assay began. Two hours post exposure, the nematodes within a 4 mm diam. around the body of the root gnat were counted. Ten EPN species/strains (4 *Steinernema* spp. and 5 strains of *H. bacteriophora*) were tested. The results indicated that 3 species/strains (two strains of *Steinernema feltiae* and one strain of *Heterorhabditis bacteriophora*) showed up to 15% attraction rate (the number of nematodes around the insect/total number of nematodes in petri dish), significantly greater than the other 7 EPN species/strains (4-10%). Moreover, the comparison of attraction of two strains of *H. bacteriophora* (Hb-NJ and Hb-Zt) to chive root alone, the root gnat alone, and both together suggested both EPN strains displayed attraction to both roots and the insect but a greater number of EPNs were attracted to the root gnat alone than to chive roots alone. No significant difference was observed in the number of EPNs attracted to both root gnat and chive root together compared with root gnat treatment alone. In addition, we found that *Heterorhabditis* and *Steinernema* spp. were attracted to pH gradients formed by acetic acid in Pluronic gel. The preferred pH range of attraction for 5 strains of *H. bacteriophora* and 2 strains of *H. megidis* were from 4.46 ± 0.07 to 5.04 ± 0.11 , and from 5.37 ± 0.07 to 6.92 ± 0.02 for 5 *Steinernema* spp. (*S. carpocapsae*, *S. feltiae*, *S. glaseri*, *S. litorale*, *S. riobrave*), indicating low pH as an attractant for *Heterorhabditis* spp., which is similar to results with root-knot nematodes, and higher pH for *Steinernema* spp. Further studies on tritrophic interactions and attractants of EPNs would provide more understanding of EPN infection mechanisms and be helpful for improving biocontrol efficacy. The results suggest that Pluronic gel is broadly applicable for the study of host or host-habitat seeking behaviors and chemotaxis of entomopathogenic nematodes.

DETERMINING NEMATICIDAL ACTIVITIES OF FUNGAL CULTURE FILTRATES OF 12 *BEAUVERIA BASSIANA* STRAINS AGAINST *MELOIDOGYNE INCOGNITA*. Li, J., J.Z. Dong, and M.J. Guan. College of Plant Protection, Agricultural University of Hebei, Baoding, Hebei, China, 071001.

Biological control potential of fungi (*Irpex lacteus*, *Aspergillus glaucus*, *Calocybe gambosa* etc.) against plant-parasitic nematodes (PPNs) has been well demonstrated. The objective of this study was to evaluate the biological activities of fungal culture filtrates of 12 *Beauveria bassiana* strains against root-knot nematode, *Meloidogyne incognita*. The strains of *B. bassiana* were transferred to shake-flasks containing improved czapek dox liquid medium and kept on the shaking table (150 rotations / minute) for 7 days at 25 °C. Using a centrifuge method, fungal culture filtrates were separated from spores and mycelium. *M. incognita* suspension was made containing 400 juveniles / 1 ml of sterile water. Then, 200 µl of each fungal culture filtrate was transferred to a petri dish containing 200 µl *M. incognita* suspension. The efficacy of fungal culture filtrates along with the control (sterile water) were compared at 27 °C in the incubator with 3 replications. The mortality and corrected mortality of *M. incognita* was determined after 24 h and 48 h by examining through microscope. NaOH stimulation method was used to determine whether nematodes died. Results showed that BD-B038-1, BD-B173, BD-B189 and BD-B1024 were

highly virulent strains against *M. incognita* with the corrected mortality of 97.52%, 68.55%, 91.63% and 78.41%, respectively, after 24 h of exposure. The corrected mortality of these four strains after 48 hours were 98.74%, 96.30%, 95.14% and 92.26%, respectively. Among all strains, the corrected mortality of BD-B038-1 was the highest, and decreased gradually with the increase in dilution times. When it was diluted by 2, 4, 8 times, the corrected mortality was 96.14%, 93.23% and 83.75% after 48 hours, respectively. When it was diluted 16 times, the corrected mortality was as low as 46.91%. When it was diluted 128 times, the corrected mortality was only 8.51%. Future research will focus on obtaining concentrated products using freeze drying technology, in order to identify the LC50 of fungal culture filtrates against *M. incognita*. Results from these studies may provide scientific basis for expanding applications of *B. bassiana* against PPNs.

CELL WALL MODIFICATION INDUCED BY RENIFORM NEMATODE IN COTTON ROOTS. Li, W.¹, P. Agudelo¹, C. Wells² and R.L. Nichols³. ¹Department of Plant and Environmental Sciences, Clemson University, Clemson, SC, 29634, ²Department of Biological Sciences, Clemson University, Clemson, SC, 29634, ³Agricultural and Environmental Research, Cotton Incorporated, Cary, NC, 27513.

The semi-endoparasitic reniform nematode (*Rotylenchulus reniformis*) is a major yield-limiting pathogen of upland cotton (*Gossypium hirsutum*). Parasitism by reniform nematode involves significant changes in plant roots, leading to the formation of multicellular feeding structures called syncytia, which are characterized by cell wall dissolution and other cellular modifications. We used a split-root system to study the involvement of cotton cell wall metabolism genes in the plant's response to reniform nematode infection. Susceptible cotton 'Deltapine 50' plants were grown with their roots equally separated into two pots, and one side was inoculated with vermiform stages of reniform nematode. At 3, 9 and 12 days after inoculation, cotton roots from both pots were sampled for RNA extraction and sequenced. Transcriptomes for each condition and time were assembled *de novo* with the Trinity pipeline. Differentially expressed (DE) genes were selected with the statistical program DESeq2 (padj<0.01), among which 30 DE genes participated in cell wall component metabolism. The potential roles of extensins-like proteins, cellulose synthases and pectin modification genes are discussed. Gene Set Enrichment Analysis was performed to identify enriched Gene Ontology (GO) terms at different infection stages (FDR<0.05). Twenty-seven cell wall GO sets were enriched in either inoculated or non-inoculated cotton root tissues. This work provides information to outline how plant cell wall genes are involved in syncytium formation, and to understand reniform nematode parasitism of upland cotton roots.

COTTON ROOT ARCHITECTURE RESPONSES TO RENIFORM NEMATODE INFECTION. Li, W.¹, P. Agudelo¹, C. Wells² and R.L. Nichols³. ¹Department of Plant and Environmental Sciences, Clemson University, Clemson, SC, 29634, ²Department of Biological Sciences, Clemson University, Clemson, SC, 29634, ³Agricultural and Environmental Research, Cotton Incorporated, Cary, NC, 27513.

Reniform nematode (*Rotylenchulus reniformis*) is a semi-endoparasitic pathogen of upland cotton (*Gossypium hirsutum*), causing significant yield loss every year. In the reniform nematode life cycle, the female penetrates plant roots, stops at one endodermal cell as the initial site, and set up a multicellular feeding structure called syncytium in pericycle. The pericycle is the outermost layer of the vascular cylinder of the root and is the site where the new lateral roots initiate. Transcriptomic data confirmed lateral root initiation genes were differentially expressed during reniform nematode infection of cotton roots, including auxin efflux carriers, auxin transporter proteins, and ethylene-responsive transcription factors. The objective of this study is to measure potential changes in root architecture caused by reniform nematode infection. We used a split-root system to separate susceptible upland cotton (Deltapine 50) roots equally into two pots and only inoculated one side with reniform nematodes. At 3, 6, 9, 12 and 18 days after inoculation, cotton roots from both inoculated and non-inoculated sides were analyzed in a root scanner. Root length, number of forks and tips, and fractal dimension were measured using the WinRHIZO software. Significant differences were observed in fractal dimension and fork density between inoculated and non-inoculated roots, with infected roots presenting more branching. Additional root scans were performed on individual cotton seedlings cultured in germination pouches to confirm the differences. This work provides insight into the effect of reniform nematode parasitism on cotton root architecture.

DIFFERENTIAL EXPRESSION OF CANDIDATE GENES FOR ANALYSIS OF RESISTANCE AND SUSCEPTIBILITY OF *COFFEA CANEPHORA* CLONES TO *MELOIDOGYNE PARANAENSIS*. de Lima, E.¹, E.C.S. Rêgo¹, M.G. Cotta², T.S. Costa², F.A. Carneiro², R.M.D.G. Carneiro³, P. Marraccini^{3,4} and A.C. Andrade². ¹Universidade de Brasília, Campus Universitário Darcy Ribeiro, Brasília, DF, Brazil 70910-900, ²Universidade Federal de Lavras, Campus Universitário, Lavras, MG, Brazil 37200-000, ³Embrapa Recursos Genéticos e Biotecnologia, Parque Estação Biológica, Brasília, DF, Brazil 70770-901, ⁴CIRAD UMR AGAP, Avenue Agropolis, Montpellier, France 34398.

Meloidogyne paranaensis is a root-knot nematode species affecting coffee crops development in several regions of Brazil. The nematode-plant interactions, as well the physiological processes of this parasitism and the coffee genes (directly or indirectly) involved in the resistance to this nematode, are poorly understood. Previous work reported that the drought-tolerant "clone 14" of *Coffea canephora* conilon was also resistant to six different populations of root-knot nematodes,

including one of *M. paranaensis*, while the drought-sensitive “clone 22” was susceptible to nematode infections. The objectives of the present study were (i) to analyze the gene expression profiles in roots of the clones 14 and 22 infected (or not) by nematodes, and (ii) to identify putative candidate genes putatively involved in nematode resistance by qPCR experiments. This was performed by extracting root total RNAs from clones 14 and 22 after 4, 8, 12, 20, 32 and 45 days after inoculation of *M. paranaensis*. As a control, RNAs of the same clones were also extracted from uninfected roots. These RNAs were then converted in cDNAs and further used in RT-qPCR experiments to check the gene expression profiles of the genes *Cc00_g16260* and *Cc10_g14530*, genes of unknown function identified in *C. canephora* genome of reference, but also of *CcCPII* (*Cc03_g09540*, GenBank accession number JF950585) gene coding for a cysteine protease inhibitor known to be involved in plant defense responses against biotic stress, and *Cc01_g13400* gene that encodes a protein phosphatase (PP2C) putatively involved in abscisic acid (ABA) signalization pathway. These results were standardized using the constitutive expression of the *CcUBQ10* gene coding for ubiquitin as a reference. For the genes *Cc00_g16260*, *Cc10_g14530* and *CcCPII*, expression profiles were up-regulated by nematode infection and higher in roots of clone 14 than in those of clone 22. Regarding the PP2C-encoding gene, its expression decreased in roots of clone 22 inoculated by *M. paranaensis* but increased upon infection in those of clone 14. Even if the ABA phytohormone is well known to be involved in plant responses to abiotic stresses (such as drought, for example), our results support the idea that ABA pathway is also involved (directly or indirectly) in the responses of the drought-tolerant clone 14 of *C. canephora* conilon to *M. paranaensis* infection, therefore suggesting that “cross-talks” between biotic and abiotic signaling pathways occurred specifically in this coffee clone.

FREQUENCY-DEPENDENT SELECTION BETWEEN *MELOIDOGYNE ARENARIA* AND *PASTEURIA PENETRANS*. Liu, C.¹ and P. Timper². ¹Department of Plant Pathology, University of Georgia, Tifton, GA 31794, ²USDA ARS, P.O. Box 748, Tifton, GA 31793.

In negative frequency-dependent selection, the parasite should adapt to the most common host genotype because a parasite genotype that can infect most hosts is favored. In turn, a rare host genotype that is resistant to the dominant parasite genotype is then favored, but after a time lag, its frequency will increase and it will become common. Subsequently the parasite should adapt to the new common genotype forming a cyclical pattern. *Pasteuria penetrans* is bacterial parasite of root-knot nematodes, *Meloidogyne* spp. Both *P. penetrans* and *Meloidogyne* spp. populations have been shown to be heterogeneous. In this study, we hypothesized that the dominant phenotype in a population of *Meloidogyne arenaria* changes over time, leading to changes in the phenotype of *P. penetrans* in the field. There is no direct way to determine the nematode phenotype in the field; therefore, we used nematode single egg mass (SEM) lines that differ in attachment phenotype as probes to test the spore phenotype. Soil samples from four replicate plots of both continuous peanut and peanut-soybean-peanut rotation were collected once per year at the Gibbs farm near Tifton, GA from 2013 to 2015. Second stage juveniles from four SEM lines were added to soil from each plot to bioassay for endospore attachment. To standardize the data, mean spore attachment in each SEM line was divided by the mean spore attachment of all SEM lines. In 8 plots and 3 years (N=24), there was one and sometimes two dominant spore phenotype in 20 out of 24 instances (83%). Changes in the dominant spore phenotype were rapid. In 8 plots from 2013 to 2014 and 2014 to 2015 (N=16), the dominant spore phenotype declined in the next year and was replaced by another dominant phenotype in 11 out of 16 instances (69%). In a few plots, we did not detect a dominant spore type. Perhaps none of the SEM lines were receptive to the dominant spore phenotype that year or each phenotype was maintained at a low equilibrium level. Our results indicate that frequency-dependent selection is occurring in a field population of *P. penetrans* and *M. arenaria*, and genetic variation is likely to be promoted. This evidence is important to understand the causes and maintenance of genetic diversity within populations of *P. penetrans* and its host *M. arenaria*.

THE FALSE ROOT-KNOT NEMATODE *NACOBBUS ABERRANS* REDUCES THE LEVEL OF COMPOUNDS ASSOCIATED WITH DEFENSE RESPONSES OF CHILLI PEPPER. López-Martínez, N.¹, J.P. Fernández-Trujillo², M. Biesaga³, and E. Zavaleta-Mejía⁴. ¹Departamento de Fitotecnia, Universidad Autónoma Chapingo. Texcoco C.P. 56230, México, ²Departamento de Ingeniería de Alimentos y Equipamiento Agrícola. Universidad Politécnica de Cartagena (UPCT). 30203. Cartagena (Murcia), ³Department of Chemistry, University of Warsaw, Pasteura 1, 02-093. Warsaw, Poland, ⁴Instituto de Fitosanidad, Colegio de Postgraduados, Montecillo, Edo. De México, C.P. 56230, México.

Resistance in chilli pepper CM334 (*Capsicum annuum* L.) against different pathogens is associated with compounds synthesized through the phenylpropanoid pathway. These compounds could be involved like physical or biochemical barriers, constitutively present or induced during infection. In this work, we determined which phenylpropanoid metabolites were altered when plants of CM334 were successfully infected by the false root-knot nematode *Nacobbus aberrans* *Nacobbus aberrans* (Thorne, 1935) Thorne & Allen, 1944. Roots were collected at 7, 14, 21 and 28 days after nematode inoculation and chemically analyzed for their lignin content and phenylpropanoid profile by quantitative lignin assay and high-performance liquid chromatography with electrospray ionization-tandem mass spectrometry (HPLC-ESI/MS). *In vitro* effect of flavonoids on second-stage juveniles (J2) of *N. aberrans* and *Meloidogyne incognita* were also tested. The lignin content was similar in all treatments excepting when *M. incognita* was involved ($P \leq 0.05$). Hidroxibenzoic (*p*-HBA), gallic,

caffeic, syringic, ferulic, vanillic and chlorogenic acids were found in the root system. Chlorogenic acid was the phenolic acid in the highest quantity in CM334 root (from 209 to 547 $\mu\text{g}\cdot\text{g}^{-1}$ of dry matter). In some sampling points, inoculated plants had lower contents of both phenolic acids and flavonoids than those non-inoculated plants (control). At all sampling dates, concentration of *p*-HBA, ferulic and chlorogenic acids was lower in inoculated plants as compared to the control ($P\leq 0.05$). Quercetin-3-O-rutinoside (rutin) was the only flavonoid found in the root system. This flavonoid had a nematocidal effect as shown in an experiment carried out on with J_2 of *N. aberrans*, whereas it only had a nemastatic effect on *M. incognita*. These results confirm that the infection by *N. aberrans* reduces the level of compounds associated with the defense responses of chilli CM334 allowing its successful establishment.

REDUCTION IN SOYBEAN PRODUCTION THROUGH EARLY INFECTION BY *HETERODERA GLYCINES* AND *MACROPHOMINA PHASEOLINA*. Lopez-Nicora, H.¹, B.W. Diers², A.E. Dorrance³, and T.L. Niblack¹. ¹Department of Plant Pathology, Ohio State University, Columbus, OH 43210, ²Department of Crop Sciences, University of Illinois, Urbana, IL 61801, ³Department of Plant Pathology, Ohio State University OARDC, Wooster, OH 44691.

Heterodera glycines, the soybean cyst nematode (SCN), is an economically important pathogen of soybean worldwide. Charcoal rot of soybean, caused by the fungus *Macrophomina phaseolina*, is affecting soybean production in areas where it has not previously been a problem. The objective of this study was to determine the effect of SCN and charcoal rot on yield and plant emergence of soybean lines with different levels of resistance to *H. glycines*. Soybean lines developed through four backcrosses (BC₄) were used, half of the lines were predicted to have the SCN resistance allele at *Rhgl* from PI 88788 based on a linked genetic marker and the other half with the susceptible allele. A standardized protocol for SCN resistance screening was used to measure the response of these lines to SCN in the greenhouse. Soybean lines were planted in 2013 and 2014 in two experimental fields infested with *H. glycines*. In a split-plot experimental design, soybean genotypes were planted in adjacent plots, one with *M. phaseolina* added pre-plant and the other without. Fifteen to twenty soil cores were collected at each plot at planting and harvest for initial (Pi) and final (Pf) *H. glycines* egg counts and *M. phaseolina* colony-forming units. Stand counts, nematode reproduction factor (RF = Pf/Pi), and soybean yield were also collected. In greenhouse studies, resistant lines infested with SCN HG Type 0 had female indices (FI) below 10 and differed from susceptible lines. Resistant lines infested with SCN HG Type 2.5.7 from the 2013 and 2014 fields had FI above 10, but still differed from susceptible lines. Soybean yield was reduced as *H. glycines* Pi increased. In 2013, SCN Pi was over 2,500 eggs/100 cm³ soil and resistant lines yielded significantly more than susceptible lines. In 2014, however, SCN Pi was over 6,500 eggs/100 cm³ soil and significant yield difference between lines was not detected. On average, *H. glycines* RF in plots planted to resistant lines were over 30% and 65% lower than plots planted to susceptible lines in 2013 and 2014, respectively. Yield was not affected by charcoal rot; however, *M. phaseolina* significantly reduced soybean emergence, contributing to reduction in soybean performance through early infection. Soybean emergence in plots infested with *M. phaseolina* was reduced by over 10% and 35% compared with uninfested plots in 2013 and 2014, respectively. Results from these studies showed that the marker alleles of lines for *Rhgl* and the greenhouse studies reliably predicted the response of soybean lines to SCN HG Type 0 and HG Type 2.5.7. In the field studies, however, the response of these lines to SCN HG Type 2.5.7 was dependent on initial population.

***HOPLOLAIMUS SMOKYI* N. SP. (HOPLOLAIMIDAE), A LANCE NEMATODE FROM THE GREAT SMOKY MOUNTAINS. Ma, X.¹, P. Agudelo¹, E. Bernard², C.M. Holguin³, and R.T. Robbins⁴.** ¹Department of Plant and Environmental Sciences, Clemson University, Clemson, SC 29634, ²Department of Entomology and Plant Pathology, University of Tennessee, Knoxville, TN 37996. ³Corpoica, Rionegro, Colombia, ⁴Department of Plant Pathology, University of Arkansas, Fayetteville, AR 72701.

During a survey of fauna of the Great Smoky Mountains National Park, along the Tennessee–North Carolina border in the southeastern United States, a *Hoplolaimus* species was isolated from a mixed forest sample of maple (*Acer* sp.), hemlock (*Tsuga* sp.) and silverbell (*Halesia carolina*). Juveniles, females and males of the lance nematode were examined for morphological characteristics, morphometrics, and phylogenetic relationships, and considered to be an undescribed species. The new species, *Hoplolaimus smokyi*, is characterized by possession of lateral field with four incisures, excretory pore posterior to hemizonid, esophageal glands with three nuclei, scutella anterior and posterior to vulva, epiptygma absent, and stylet length 44 to 51 μm . Morphologically, *H. smokyi* is closest to *H. galeatus* and *H. stephanus*, and could be considered a cryptic species that can be distinguished only by minor morphological differences like the 5-6 annules in the lip region (compared to 4 in *H. stephanus* and 5 in *H. galeatus*) and morphometric values (average body length $\sim 15\%$ larger than *H. stephanus* and $\sim 12\%$ smaller than *H. galeatus*). However, *H. smokyi* is phylogenetically unique. The phylogenetic analysis based on ribosomal and mitochondrial gene sequences suggests a lineage distinct from *H. galeatus* and *H. stephanus* (Bayesian posterior probability 100%) and separate from other *Hoplolaimus* species like *H. columbus* and *H. magnistylus*. This new species is described with morphometric data on adults of both sexes, and phylogenetic relationships with other lance nematode species present in the southeastern United States are discussed.

ADAPTATIONS OF PLANT-PARASITIC NEMATODES TO EXTREME CONDITIONS IN AGRICULTURAL FIELDS - MANIPULATING THE SOIL ENVIRONMENT TO FOIL NEMATODE PESTS. **MacGuidwin, A.E.**, Department of Plant Pathology, University of Wisconsin-Madison, Madison, WI 53706.

Pest nematodes in agricultural fields are incredibly tenacious, persisting in a soil environment prone to turbulence and extreme fluctuations of moisture and temperature. Winters in many regions are brutal with soil freezing to depths well beyond the dispersal capacity of microscopic nematodes. Adaptations to freezing are relatively rapid and, in most cases, result in survival rates that insure populations persist. Multiple mechanisms confer tolerance of freezing, including cryoprotective dehydration. Laboratory studies showed that the freezing tolerance of *Meloidogyne hapla* was increased in dry soil and this finding has been purposed as a recommendation for irrigating fields in the fall to diminish nematode survival. Tolerance of desiccation is also an advantage during the growing season, particularly in systems with frequent tillage events. *Pratylenchus penetrans*, a species highly tolerant of freezing and desiccation, is a common pest in the northern U. S. with a wide host range. In our lab, this species survived in a dehydrated state up to two years in an agar medium and six years in stored soil. In collaboration with farmers, we conducted a proof-of-concept study to demonstrate the value of combining heat with moisture for killing *P. penetrans* in the field. These and other examples revealed that research about adaptations of pest nematodes to temperature and moisture extremes is of interest to farmers and has potentially useful applications.

NEMATODE COMMUNITY ANALYSES UNDER NO-TILL AND CONVENTIONAL TILLAGE WITH DIFFERENT CROP ROTATIONS IN A LONG TERM EXPERIMENT IN SOUTH BRAZIL. **Machado, A.C.Z., S.A. Silva, and O.F. Dorigo.** IAPAR, 86047-902, Londrina, Paraná State, Brazil.

Composition and abundance of the nematode fauna have been used as soil health indicators in many different environments, because nematodes are functionally diverse and ubiquitous and respond readily to environmental changes. Tillage and cropping patterns cause profound changes in populations of soil nematodes. Besides, the community of soil organisms is dependent primarily on autotrophic input from plants or on subsidiary input from other sources and responds in characteristic ways to enrichment of its environment by organic matter. Nematode faunal analyses are providing potential tools for soil web management. So, we analyzed the nematode community composition in a long term experiment, in a clayey oxisol (72% clay), in South Brazil, with different winter cover crop species and two soil managements systems (no-till and conventional tillage). Soil was sampled in April 2015, taken at 0-20 cm depth. Nematodes were extracted from a subsample of 50 cm³ using the Baermann Funnel method. 100 nematodes from each sample were identified to genus or family under optical microscope. Nematodes were classified by trophic habit and by colonizer-persister (cp) groups. Analyses were performed using NINJA (<https://seriebriennikov.shinyapps.io/ninja/>). Cropping pattern, tillage system and cover crops affected the composition of the nematode fauna and food web indices. In the conventional tillage, there are a predominance of semi-endoparasitic nematode species, followed by the omnivores. In relation to structure index, values showed that all experimental units were considered mature, nitrogen-enriched, with a low C:N relation and with a predominantly bacterial via for organic matter decomposition. In the no tillage system, there are a predominance of fungivores and omnivores nematode species and, in relation to structure index, values showed that the majority of the experimental units were considered mature, nitrogen-enriched, with a low C:N relation and with a predominantly bacterial via for organic matter decomposition. Crop rotation with cover crops, despite the cropping system used, has a positive and durable effect in the soil nematode communities, which leads to a more diverse and ecologically mature environment, with lower environmental disturbance. These benefits certainly could reflect the soil health quality provided by the crop rotation.

MOLECULAR CHARACTERIZATION AND PHYLOGENY OF *DITYLENCHUS* FROM *CIRSIIUM ARVENSE* IN THE CANADIAN PRAIRIES. **Madani, M. and M. Tenuta.** Department of Soil Science, University of Manitoba, Winnipeg, MB, Canada R3T 2N2.

The stem and seed nematode of creeping thistle (*Cirsium arvense*), *Ditylenchus weischeri*, was first described in 2011 in Russia based on morphological and molecular (ITS-RFLP, *hsp90* sequence) differences of the nematode from *D. dipsaci*. More recently, we reported creeping thistle in commercial fields from the provinces of Saskatchewan and Manitoba parasitized by *D. weischeri*. Here we present a detailed phylogenetic position of the nematode in relation to other *Ditylenchus* species based on molecular analyses. *Ditylenchus weischeri* from creeping thistle plants and seeds contaminating yellow pea grain samples from Canada and creeping thistle from Russia were examined. Garlic infested with *D. dipsaci* from Quebec and sequence data of other species of *Ditylenchus* retrieved from the Genbank database was also used. The Canadian *D. weischeri*, showed minor differences in morphology to the holotype type of this species from Russia. Sequences of the Internal Transcribed Spacer (ITS) region, D2-D3 expansion region of the 28S gene and heat shock gene (*hsp90*) were used to construct individual dendrograms of relatedness of *Ditylenchus* species. For each of the three genes examined, *D. weischeri* grouped separately from other *Ditylenchus* species. These results provide multiple lines of evidence that *D. weischeri* is not only molecularly distinct from *D. dipsaci* but also other species of *Ditylenchus*.

DUPLEX CONVENTIONAL PCR AND REAL TIME PCR MELTING CURVE ANALYSIS OF ONTARIO POPULATIONS OF SOYBEAN CYST NEMATODE, *HETERODERA GLYCINES* ICHINOHE. **Madani, M.¹, M. Tenuta¹, A. Tenuta², and T. Welacky³.** ¹Department of Soil Science, University of Manitoba, Winnipeg, MB, Canada R3T 2N2, ²Ontario Ministry of Agriculture, Food and Rural Affairs, University of Guelph Ridgetown Campus, PO Box 400, Ridgetown, ON, Canada N0P 2C0, ³Greenhouse and Processing Crops Research Centre, Agriculture and Agri-Food Canada, Harrow, ON, Canada N0R 1G0.

The Soybean Cyst Nematode (SCN), *Heterodera glycines*, is an import pest of soybean limiting yields in Ontario and other soybean production areas worldwide. In this study, we evaluated the suitability of two published, ITS and SCAR derived species specific primers sets and three new mitochondrial DNA primer sets for the CoxII, CoxIII, ND4 genes for identification of *H. glycines* from commercial fields in Ontario. *Heterodera glycines* individuals from 20 commercial fields from Ontario were examined. As well, samples of *H. glycines* from two states in the USA and negative controls of two other species of *Heterodera* (*H. schachtii*, *H. carotae*) and two plant parasitic nematode species for each of the genera *Ditylenchus* and *Meloidogyne* were also examined. The two published primer sets resulted in 15% of the Ontario samples having failed, very weak, or inconsistent PCR amplification, while the *H. glycines* samples from the USA were positive. The primer sets were not specific for *H. glycines*, having cross reacted with *H. trifolii*. The mitochondrial DNA sequence of *H. glycines* from Genbank was used to construct three new primer sets specific for *H. glycines* based on cytochrome c oxidase subunit II (CoxII), Cox III and ND4 genes. Primer set CoxII generated a single band of 322-bp length using conventional PCR for all samples from Ontario and *H. glycines* from the USA. The primer sets, ND4 and CoxIII, were then examined in duplex conventional and real time PCR melting curve analysis, respectively, using the D3S/D3B region of the 28s rDNA gene. With duplex conventional PCR of all *H. glycines* samples from Ontario and the USA, the D3A/D3B and ND4 primer sets resulted in a 638-bp and 345-bp length amplicon, respectively. With real time PCR melting curve analysis, the D3A/D3B and CoxIII primer sets resulted in melting temperature of (T_m) 86.0 ± 0.2 and 73.5 ± 0.5 °C, respectively, for one or many J2 individuals in a reaction for samples from Ontario and the USA. All non *H. glycines* species of nematodes tested did not result in amplicons with the CoxIII and ND4 primer sets. The conventional PCR and real time assays developed here provide a more accurate and faster method of identification of *H. glycines* from Ontario than currently available.

NEMATODOS ASOCIADOS AL CULTIVO DE CAÑIHUA (*CHENOPODIUM PALLIDICAULE* AELLEN.) DE LA REGIÓN PUNO. (NEMATODES ASSOCIATED CULTIVATION OF CAÑIHUA (*CHENOPODIUM PALLIDICAULE* AELLEN.) IN REGION OF PUNO.) **Mamani Cano, Z.D.¹, L.M. Israel², F.C. Yeni Felipa², A.G. Marilia², G.A. Sthewart Irwin¹, C. J. Shadam Elvis¹, Z.T. Noely Carelim¹, and B.P. Rosario Ysabel².** ¹Universidad Nacional del Altiplano Puno, Perú. Facultad de Ciencias biológicas¹, Facultad de Ciencias Agrarias².

El cultivo de cañihua es considerado un producto en la seguridad alimentaria del poblador andino, no en tanto plagas y enfermedades limitan el cultivo. Dentro de estos los nematodos fitoparásitos, quienes atacan sin dar señales de sintomatología, no obstante pueden llegar a impedir el paso de nutrientes y el normal crecimiento de la planta, ocasionando pérdidas en la producción del cultivo. El objetivo del presente estudio fue identificar los géneros de nematodos fitoparásitos asociados al cultivo de cañihua de la región de Puno-Perú. Se realizó una recolección de muestras de suelo en los distritos de Lampa, Pucará, Orurillo e Ilave. La extracción de nematodos fue realizada por el método de fluctuación centrífuga en solución Sacarosa. Se identificaron seis géneros de nematodos fitoparásitos, los cuales fueron *Globodera* spp., *Helicotylenchus* spp., *Mesocriconema* spp., *Xiphinema* spp., *Dorylaimus* spp., *Nacobbus* spp. y nematodos de vida libre (96.3; 41.8; 17.6; 14.2; 5.0; 5.6; 92.1% respectivamente). Cabe mencionar que el género de mayor presencia en el cultivo de la cañihua es *Globodera* spp.

NACOBBUS ABERRANS: SOLVING A GORDIAN KNOT? **Manzanilla-López, R.H.¹, N. López-Martínez², and A. Tovar-Soto³.** ¹16 Coleswood Road, Harpenden, AL5 1EQ, UK, ²Departamento de Fitotecnia, Universidad Autónoma Chapingo. Texcoco C.P. 56230, México, ³Escuela Nacional de Ciencias Biológicas, Carpio y Plan de Ayala, México D.F., Col. Sto. Tomás, C.P. 11340.

Nacobbus aberrans sensu lato is one of the most important nematode pests affecting crops such as tomato, chilli pepper, beans, sugar beet and potato in both North America and South America. It is currently accepted that *Nacobbus aberrans s. l.* is a species complex. Although data from molecular approaches (RAPD, ITS, RFLP, D2-D3-rRNA, mt DNA, ISSR) is supportive of the existence of cryptic species within the complex, this has yet to be widely accepted at the taxon level, despite the fact that a number of distinct clades have been consistently demonstrated – the ‘Gordian knot’. Indeed, not only are these clades not accepted at species level, they are rarely considered when doing field trials or assessing management strategies. It is clear, therefore, that much *Nacobbus* research in Latin America is simplistically based on populations that are essentially uncharacterized, cryptic speciation/variation in the target organism being ignored or overlooked. The impact of this situation has yet to be adequately defined as few studies on, for example host range, susceptibility to nematophagous fungi, etc., have been done on adequately (by which we mean molecularly) characterized populations of *Nacobbus*. This is clearly an extremely unsatisfactory situation for designing local management strategies as more than one clade may be present in the soil. In addition, if molecular clades can be tied to host range, there is the added complication of establishing and enforcing

appropriate quarantine regulations. Progress has been made in understanding, at biochemical level, the interaction between *N. aberrans* with other pathogens in affecting host resistance, thereby revealing the fascinating complexity of this nematode and its survival strategies. Biological control with microorganisms such as *Pochonia chlamydosporia*, has started to be explored but only a few isolates belonging to the *Nacobbus* biotype have been isolated from nematode eggs and we do not yet know if a *Nacobbus* serine protease VCP1 polymorphism associated with host preference exists, as in cyst and root-knot nematodes. Research on *Nacobbus* spp. is usually done in Latin American countries, mostly universities, and where no national projects exist to give continuity to research deserving of further exploration and development to develop management strategies and phytosanitary regulations. One result of this uncoordinated approach is the relentless dissemination of the nematode to previously clean areas. The lack of research funding on *Nacobbus* partly supports, *de facto*, the *status quo* of there being only two valid species: *Nacobbus dorsalis* and *N. aberrans sensu* Sher – despite much evidence to the contrary. If we do not know what we are working with, the implication is that much of the research so far conducted on *Nacobbus* will have to be discarded. Is it possible to solve the *Nacobbus* Gordian knot, thereby opening the door to improved management?

HOST SUITABILITY OF HOT PEPPER GENOTYPES TO *MELOIDOGYNE ENTEROLOBII*. Marques, M.L.S.¹, F.A. Carneiro², J.O. Silva², R. A. Teixeira², A. R. Nascimento², and M.R. Da Rocha². GO ¹Instituto Federal Goiano Campus Ceres, Departamento de Ciências Agrárias, Rodovia GO 154 Km 3, Caixa Postal 51, CEP 76300-000, Ceres-GO. ²Escola de Agronomia, Universidade Federal de Goiás, Campus Samambaia, Avenida Esperança, s/n, CEP 74690-900, Goiânia- GO.

The root knot nematode *Meloidogyne enterolobii* (= *sin. M. mayaguensis*) was first found in Brazil parasitizing guava trees (*Psidium guajava* L.). It has been found also parasitizing vegetables, such as tomato and peppers, including cultivars with resistance to *M. incognita* e *M. javanica*. Therefore, the identification of sources of resistance to *M. enterolobii* is now one of the challenges to achieve a sustainable management. The present study has the objective to evaluate the host suitability of hot pepper genotypes (*Capsicum* spp.) to *M. enterolobii*. We tested 54 genotypes in a completely randomized design, with 10 replications, under greenhouse conditions. The inoculum was obtained from a field of guava trees and multiplied under greenhouse on eggplant (*Solanum melongena*) cv. Napoli. Seedlings of the 54 genotypes were prepared using styrofoam tray filled with coconut fiber as a substrate. When seedlings had 2 to 4 pairs of leaves they were transplanted to plastic bags (2L) containing pre autoclaved mixture of sand and soil (2:1). The inoculation was performed 15 days after transplanting using a suspension of 4.000 eggs + J2 per seedling. The evaluations were made at 90 days after inoculation by extracting the nematodes from roots. The reproduction factor (RF) was calculated dividing the final population by the initial population (RF = Pf/Pi). Data were analyzed statistically and the means compared by the Scott-Knott test ($p < 0.05$). Plants with RF > 1 were considered as susceptible and RF < 1, as resistant. From the 54 genotypes tested 19 were resistant to *M. enterolobii* with the RF ranging from 0.02 to 0.66. 35 genotypes were considered susceptible with RF ranging from 2.0 to 32.92.

UNDERSTANDING THE ROLES OF SOIL WATER CONSERVATION ON INDIGENOUS ENTOMOPATHOGENIC NEMATODES AND ENTOMOPATHOGENIC FUNGI IN A NO-TILL ORGANIC MULCHING SYSTEM. Marques, J., K.-H. Wang, B.S. Sipes, and Z. Cheng. Department of Plant and Environmental Protection Sciences, University of Hawaii, Manoa, HI 96822.

Restrictions on synthetic pesticide use has prompted farmers to look for alternative insect pest management strategies. Entomopathogenic nematodes (EPN) and entomopathogenic fungi (EPF) are promising biological control agents for insect pests with a life cycle in the soil. Use of EPN and EPF in the Hawaiian Islands are challenged by quarantine regulations and the failure to adapt and persistent in the field when introduced using an augmentative approach. This research focused on providing a favorable soil environment through soil water conservation that can enhance indigenous EPN and EPF populations, otherwise known as conservational biological control. It is hypothesized that cover cropping followed by a no-till practice in a Hawaiian Oxisol would provide a more favorable environment via organic mulch, reduced soil disturbance, and improved water conservation, thus increasing population densities and effectiveness of EPN and EPF. A field trial was conducted to compare pre-plant treatment effects of 1) black oat (*Avena strigosa*) as cover crop in a 7-year no-till (NT), 2) bare ground (BG) followed by conventional tillage, and 3) conventional tillage followed by soil solarization (Sol) on relative abundance of EPN and EPF using a trap method with mealworm larva (*Tenebrio molitor*) at 1, 2, and 3 months after corn (*Zea mays*) planting. Gravimetric soil moisture content measurements indicated that NT had higher soil moisture content ($P < 0.05$) than BG and Sol. NT also had the fastest water infiltration rate among treatments, significantly faster than that in Sol ($P < 0.05$) suggesting improved water absorption. Although there was no difference in relative abundance of EPN and EPF among treatments at the first and third month after corn planting, a higher abundance of EPF was detected in NT than BG and Sol ($P < 0.01$) at 2 months after corn planting. However, EPN infections decreased as corn aged, especially in NT. It is likely that fungi antagonistic to EPN such as nematode-trapping fungi could have been enhanced in NT leading to a reduction of EPN. None-the-less, corn had higher tissue nitrogen and chlorophyll content ($P < 0.01$), and was taller in NT than BG and Sol ($P < 0.05$), supporting the theory that a no-till practice is a viable option for growers. Research is on-going to investigate various soil edaphic factors that might interfere with population densities of EPN and EPF in a NT cropping system.

SOIL NEMATODES AND SOIL ELEMENTAL PROPERTIES IN WHEAT PLOTS: AN ASSOCIATIVE VIEW. **Matute, M.¹, A. H. Carter² and J. Sherman³.** ¹Department of Natural Sciences, Pulaski Technical College, North Little Rock, AR 72118, ²Winter Wheat Breeding and Genetics, Washington State University, WA 99164, ³Plant Sciences and Plant Pathology Department, Montana State University, Bozeman, MT 59717.

In an investigation of soil nematodes associated with wheat (*Triticum aestivum*), we hypothesized that a nutrient rich soil will correspondingly increase soil nematode populations. We thus attempted to associate in a correlative manner the ecological functional groups of nematodes recovered from wheat soils with the major soil elements needed for plant growth (N, P, K, and S). The study was conducted in Almoda, WA. The plots were located on a clay soil with an alkaline harvest pH of 8.9. Soil samples were collected preplant and at harvest using a foot-driven soil sampler. Ten rows were randomly sampled and each sample was a composite of 6 to 8 cores collected along the row. Nematodes were extracted and soil concentration of N, P, K, and S were measured. The nematodes recovered were identified based on their morphology and classified based on their trophic guild: soil fertility enhancing nematodes (SFEN) i.e. the bacterivores and fungivores (Ba₁, Ba₂, and Fu₂) dominated by the Rhabditidae and Panagrolaimidae, Cephalobidae and Plectidae, and the Aphelenchidae and Aphelenchoididae; pest suppressive nematodes (PSN) i.e. the predators or carnivores and omnivores (Ca₄ and Om₄) dominated by Dorylaimidae, Qudsianematidae and Mononchidae; and plant-parasitic nematodes (PPN) i.e. plant tissue feeders (P₂₋₃) dominated by the Heteroderidae and Hoplolaimidae, and the Tylenchidae and Psilenchidae. At wheat harvest, levels of N (+67.5%) and S (+228%) increased as compared to the preplant levels, while K (-4.1%) and P (-9%) levels decreased. Among the nematode feeding groups, the Ca₄ (+2,155%) and the Om₄ (+575%) increased the most, followed by the Ba₁ (+142.5%) and P₃ (+115.2%). Conversely, the Ba₂ (-62.24%), followed by the P₂ (-47.4%) and Fu₂ (-27.6%) feeding groups decreased at wheat harvest as compared to preplant levels. While the Ca₄, Om₄, Ba₁, and P₃ nematode trophic groups were positively correlated with soil levels of S and N, they were negatively correlated with P and K. On the other hand, the Ba₂, P₂, and Fu₂ nematode feeding groups, were positively correlated with P and K and negatively correlated with N and S. The nematode soil ecological functions-Maturity Index (MI 2-5: +17.3%), Plant-Parasite Index (PPI: +17.3%), Enrichment Index (EI: +236.86%), and Structure Index (SI: +1,011.59%) all increased at harvest as respectively indicated. These nematode ecological soil functions were thus positively correlated with soil N and S, while being negatively correlated with soil P and K, as were the Ca₄, Om₄, Ba₁, and P₃ nematode trophic guilds. In summary, nematodes that are indicators of basal and nutrient poor soil conditions (Ba₂, Fu₂, and P₂), were negatively correlated with N and S and positively correlated with K and P. The reverse was true for the other nematode groups recovered.

ENHANCED AWARENESS OF NEMATOLOGY: EDUCATIONAL MATERIALS, EXTENSION ACTIVITIES AND SOCIAL MEDIA. **McGawley, E.C.¹, C. Overstreet¹, and A. M. Skantar².** ¹LSU AgCenter Dept. of Plant Pathology and Crop Physiology, Baton Rouge, LA 70803, ²USDA-ARS, 10300 Baltimore Avenue, Beltsville, MD, 20705.

In the past 5 years, a range of materials, groups and activities have been designed to create a visual, collaborative and interactive experience for students, faculty and crop production clientele interested in nematodes. Small (45X30cm) and large (90X60cm) format black and white and colorized posters include “Nematode Anatomy and Morphology” and “Common Genera of Plant-Parasitic Nematodes”, multimedia productions entitled “Introduction to Nematodes” (with over 5,000 downloads worldwide and available in 7 languages) and “History of The Society of Nematologists” (in English with over 300 downloads) have been produced as well as a 100 page PowerPoint presentation containing hundreds of graphics and illustrations. All of these materials are available for download (free of charge for educational purposes) from the website of the Society of Nematologists, SON, (nematologists.org). In 2014, an informal international collaboration called NEMA (Nematological Education Materials Alliance) was organized and now includes 51 members in 18 countries. Members of NEMA exchange course syllabi, lab exercises, photographs, illustrations, videos and ideas. Extension activities to educate the public have been rapidly evolving during the past decade. Information is disseminated through the use of websites, newsletters, webinars, and blogs. Publications are accessible online from most states with information about current nematode problems. Website are being developed that are more mobile-friendly to allow access to information from a variety of devices. Extension personnel are also using crop-specific text message groups to provide timely information to producers. Ask an Expert with eXtension have answered hundreds of question about nematodes from Cooperative Extension/University staff. In the first year of existence, the new SON Facebook page (facebook.com/Society-of-Nematologists-346734898856221) has 471 likes with followers from 45 countries, and the Twitter feed (@SON_nemaweb) has 198 followers from 27 countries. Our social media feeds increase connectivity among members and reach out to many outside our society who share nematological interests. We interact with the *C. elegans* community through the news feeds of the Genetics Society of America, and with other nematologists through ESN, ONTA, ICN, and Helminthological Society of Washington. Our social media activities have answered questions, pointed our followers to possible mentors, training opportunities, and jobs. Additionally, social media have increased the reach of the Journal of Nematology through posting direct links to every paper published since the September 2015 issue. SON also has a LinkedIn page to disseminate announcements and facilitate discussion for those who prefer this professional network site.

EVALUATION OF ENDEMIC POPULATIONS OF *ROTYLENCHULUS RENIFORMIS* WITHIN LOUISIANA ON SOYBEAN GENOTYPES WITH KNOWN LEVELS OF RESISTANCE TO SOYBEAN CYST NEMATODE. **McInnes, B.¹, M. Kularathna¹, E.C. McGawley², and C. Overstreet².** ^{1,2}LSU Agcenter Dept. of Plant Pathology and Crop Physiology, Baton Rouge, LA 70803.

Variation among geographic isolates of *Rotylenchulus reniformis* (reniform nematode) has been demonstrated both inter- and intrastate in the USA. A greenhouse study was conducted to evaluate sources of resistance present in soybean genotypes used in the soybean cyst nematode “HG Type” test (Pickett, PI 88788, PI 90763, PI 437654, PI 209332, PI 89772, Cloud, Lee 74) using five geographic isolates (from Ouachita, Rapides, East Carroll, Tensas, and Catahoula parishes of Louisiana) of *R. reniformis*. Experiments, conducted under greenhouse conditions, were established as an $8 \times 5 \times 4$ design representing the eight “HG Type” genotypes \times five reniform nematode isolates \times four replications. After 31 days, egg masses were stained and numbers present on a 2-g subsample were enumerated at 40x using a dissecting microscope. Vermiform life stages of *R. reniformis* in soil were extracted from a 250 g subsample of soil. Data was analyzed using analysis of variance (ANOVA) for a factorial design using “Fit Y by X” module of SAS JMP Pro, version 12.0 (SAS Institute; Cary, NC). PI 90763 and PI 89772 showed significant variation in numbers of vermiform life stages in soil across all five reniform nematode isolates. Reproduction of the nematode was greatest on Lee 74 and least on PI 90763 and PI 89772. Results add further support to the hypothesis of virulence phenotypes within Louisiana and among major southern cotton and soybean producing states. Current research is focused on the development of a greenhouse based differential host assay for *R. reniformis* and evaluation of a laboratory based assay in employing a soilless growth-pouch environment.

A CENTURY-OLD FERTILIZER PLUS NEW IRRIGATION TACTICS EQUALS A ‘GREENER’, NON-FUMING BIOCIDES. **McKenry, M.** Nematology Dept. UC Riverside, Riverside, CA 92521.

Establishing new orchards or vineyards without soil fumigation requires attention to pathogenic nematode and root rejection components of the replant problem; the latter component by switching to a genetically different plant species. The “Starve & Switch” strategy can be difficult to achieve but after ten years of searching examples are now available within three different perennial crop systems. Basically, we kill the previous tree with herbicide before removal, wait a full year and then switch to a rootstock of different species that also has nematode resistance. Six rootstocks to switch to among Vitis, Juglans and Prunus are available. Except for Juglans, existing dead roots often provide refuge for nematode life stages within old roots. Herein we report for the first time a totally soluble chemical having short half-life. This Category III product is further diluted 250 to 500-fold just before drenching into soil. Higher dosages listed above are associated with deeper soil delivery. When replanting trees and vines into drip-applied or drench-treated plant-site basins, the biological activity of the biocide is adequate to promote extra first-year growth while also protecting resistance mechanisms in first-year roots from excessive nematode feeding. While emulsifiers do not seem to be necessary for treatment efficacy, it is critical that the material is evenly administered with every drop of water. Remnant live root systems can be partially or completely killed in settings where entire root systems are reached and systemic uptake of the biocide occurs. Information is still necessary on how to implement this material in the orchard renewal process: use it for killing the old trees and replant after one year of fallow or fallow a full year and then apply the material as a pre-plant biocide. Availability of this new biocide coupled with availability of the only post-plant nematicide that can reach deeply via roots should encourage greater use of Starve & Switch strategies among perennial crops as an alternative where soil fumigation is no longer possible.

MOTU SOIL NEMATODE ASSESSMENT OF VEGETATION TYPES OF CENTRAL MEXICO. **Mejía-Madrid, H.H.** Laboratorio de Ecología y Sistemática de Microartrópodos Departamento de Ecología y Recursos Naturales, Facultad de Ciencias, UNAM. México City C.P. 04510, Mexico.

Molecular operational taxonomic unit (MOTU) methods were applied to a survey of soil nematodes recovered from a geographically broad area of Mexico that included four of the seven recognized vegetation types present in mid and eastern-central Mexico. Eleven sampling sites from eight localities that spread across de TMVB were sampled. Soils were sampled from tropical rainforest, tropical dry deciduous forest, temperate coniferous forest, and xerophytic shrub during 2013, 2014 and 2015. Aforementioned vegetation types were sampled for soil nematodes in areas that have been protected for *ca.* > 20 years and others that have been cultivated since pre-Columbian times. The range of altitudes sampled was 113 to 2400 m. Special care was taken that samples were drawn from conserved and cultivated plots within an area ≥ 30 m². The D2D3 expansion region of LSU was the main marker used because of its relative advantages over SSU. A total of 87,795 individuals of at least 20 identified families of nematodes were collected. From these, 77 high-quality sequences from individuals from different families were successfully Sanger amplified for taxonomic barcoding and identified with morphological traits. Sequences amounted to a total of 48 unique MOTU, where 100% were unique to tropical rainforest from both conserved and managed sites, 67% to tropical dry deciduous forest, 44-63% to temperate coniferous forest, 20-44% to recovering temperate coniferous forest, and between 67-100% to xerophytic shrub. Especially the latter three regions had

different degrees of land use, i.e., were crops or were abandoned terrains but former cultivars or orchards. Additionally, 43% MOTU were shared among localities and 57% exhibited an apparently locality-limited distribution. Diversity of MOTU shows that there is a wide range of probability of finding new species of soil nematodes in the regions comprising the aforementioned vegetation types, especially those conserved or recovering from agricultural practices. In general, most of the conserved temperate coniferous forest MOTU exhibited more perfect matches (99%) with sequences in GenBank, xerophytic shrub had only one match whereas all sequences from tropical rainforest and tropical deciduous forest localities rendered only unique MOTU.

AN OVERVIEW ON FORTUNIANA ROSE RESISTANCE TO ROOT-KNOT NEMATODES. Mendes, M.L. and D.W. Dickson. Entomology and Nematology Department, University of Florida, Gainesville, FL 32611.

Fortuniana rose (*Rosa fortuniana*) is used extensively as rootstock in the southeastern United States to ward off root-knot disease. It has a vigorous root system and has been reported to be resistance to stem dieback, crown gall, *Pythium*, *Phytophthora*, *Rhizoctonia*, and nematodes. However, no specific nematode has been named associated with the rootstock except *Meloidogyne hapla*. The objective of this study was to evaluate the resistance of Fortuniana rose to six root-knot nematodes commonly reported in Florida, *M. arenaria* race 1, *M. enterolobii*, *M. floridensis*, *M. hapla*, *M. incognita* race 3, and *M. javanica* race 1. The experimental design was a complete randomized block with five replicates. Each nematode species was inoculated at a density of 5,000 eggs and/or second-stage juveniles per pot. Tomato cv. AgriSet 334 was included as a susceptible host for all six nematode species. The plants were maintained in a greenhouse for 85 days, than were uprooted and evaluated for nematode galling and reproduction. The fresh root weight was also determined. Fortuniana rose was resistant to all six nematode isolates although *M. hapla* and *M. javanica* race 1 produced a few small galls. *M. hapla* did reproduce (472 eggs/g of root), but the reproduction factor was low (RF = 0.20). On tomato, the average gall index was 100 for all isolates, and the species with the lowest RF was *M. hapla* (RF = 7.80).

HOST SUITABILITY OF COFFEEWEED TO ROOT-KNOT NEMATODES. Mendes, M.L. and D.W. Dickson. Entomology and Nematology Department, University of Florida, Gainesville, FL 32611.

Coffeeweed or coffee senna (*Senna occidentalis* syn. *Cassia occidentalis*) is widespread in warm areas of the world except for Australia. In the United States it can be found in open woodlands and in disturbed areas from the Great Lakes region through to the southeastern USA. In Florida it is commonly found in peanut and cotton plantings. Although it is found throughout the world there is no information regarding its relationship with plant-parasitic nematodes. Our objective was to determine the host suitability of coffeeweed to six common root-knot nematodes reported in Florida: *Meloidogyne arenaria* race 1, *M. enterolobii*, *M. floridensis*, *M. hapla*, *M. incognita* race 3, and *M. javanica* race 1. Four weeks old coffeeweed seedlings were inoculated with 5,000 eggs and/or second-stage juveniles of each root-knot nematode species. Tomato cv. AgriSet 334 was included as a susceptible host for all six nematodes. The inoculated plants were maintained in a greenhouse for 85 days after which the plants were uprooted and evaluated for nematode galling and reproduction. The fresh root weight was also recorded. Only *M. enterolobii* and *M. incognita* reproduced on coffeeweed. *M. enterolobii* produced higher number of eggs/g of root (184 eggs/g of root) and a higher reproduction factor (RF = 1.78) than *M. incognita* (48 eggs/g of root; RF = 0.31). All nematode species reproduced well on tomato; the average gall index was 100 for all isolates, and the nematode species with the lowest RF was *M. hapla* (RF = 7.80).

YELLOW AND PURPLE NUTSEDGE AS HOSTS OF COMMUM ROOT-KNOT NEMATODES IN FLORIDA. Mendes, M.L., W.T. Crow, and D.W. Dickson. Entomology and Nematology Department, University of Florida, Gainesville, FL 32611.

Yellow (*Cyperus esculentus*) and purple nutsedges (*C. rotundus*) are common weeds in vegetable production in Florida. They are considered as the world's worst weed problem. Yet, little is known about their ability to host plant-parasitic nematodes, mainly root-knot nematodes. Our objective was to determine the host suitability of yellow and purple nutsedges to six root-knot nematode species known to occur in Florida; namely, *Meloidogyne arenaria* race 1, *M. enterolobii*, *M. floridensis*, *M. hapla*, *M. incognita* race 3, and *M. javanica* race 1. The experimental design was a complete randomized block with five replications. The test plants were inoculated with 5,000 eggs and/or second-stage juveniles of each nematode species. Tomato cv. AgriSet 334 was included as a susceptible host for all six species. The plants were maintained in a greenhouse for 85 days after which nematode galling and reproduction were assessed along with fresh root weights. Both yellow and purple nutsedges were hosts for all species tested, but only *M. hapla* and *M. javanica* race 1 induced noticeable galls on yellow nutsedge. No galls were visible on purple nutsedge. All species, except *M. arenaria*, produced a higher number of eggs per gram of root and a higher reproduction factor (RF = Pf/Pi) on yellow nutsedge than on purple nutsedge. On yellow nutsedge *M. javanica*, *M. floridensis* and *M. hapla* produced higher number of eggs, whereas *M. arenaria*, *M. floridensis* and *M. javanica* were the species that reproduced at a greater rate on purple nutsedge. In summary, nutsedges serve as a host of the major agriculturally important root-knot nematode species.

WHAT DO SOIL PROPERTY AND NEMATODE ASSEMBLAGE ANALYSES SUGGEST ABOUT INTEGRATED SOIL HEALTH MANAGEMENT IN MICHIGAN AGRICULTURE? **Mennan, S.^{1, 6}, J. Gronseth², P.D. Reeb³, A.J.M. Smucker⁴, A. Adelaja⁵, J. Warbach⁵, J. Qi², and H. Melakeberhan¹.** ¹Agricultural Nematology Laboratory, Department of Horticulture, ²Center for Global Change Education and Observation, ³Statistical Consulting Center, College of Agriculture and Natural Resources, ⁴Department of Plant Soil and Microbial Sciences, and ⁵Land Policy Institute, Michigan State University, East Lansing, MI 48824, ⁶Ondokuz Mayıs University, 55139 Samsun, Turkey.

There is an increasing emphasis on developing integrated and scalable soil health management strategies in the diverse Michigan agriculture system. In these systems, plant-parasitic nematodes are among the biotic and abiotic yield-limiting factors, and agricultural inputs (fertilizer, nematicides/pesticides and etc.) are applied up to four times per season to deal with yield limiting factors. Among other things, achieving an all-around integrated and scalable soil health management strategy requires an understanding of the agroecosystem complexes. For example, the agricultural landscape in Michigan encompasses six soil groups (orders) scattered mainly in two temperature zones, 40.1-45.0 °F (northern half) and 45.1-50.0 °F (southern half). Agricultural inputs are generally applied to address the yield-limiting factor(s) with little regard to soil group and/or temperature zones. Both soil group and temperature affect biological and physiochemical processes that, in turn, influence soil health-driven ecosystem services. Thus, understanding whether or not the same soil groups have similar or different biological structure in different temperature zone is critical to developing scalable soil health management strategies. Using nematode community and soil physiochemical analyses as indicators, the objective of this study was to determine if Udalfs, Psamments and Saprists soil sub-orders (major agricultural soils with contrasting properties) within the two temperature zones have similar or different biological functions. Soil samples were collected from natural (pristine forests and other vegetation) and disturbed (agricultural soils with altered biological functions and soil nutrients) landscapes in the northeast and southwest regions which are separated by about 300 miles. Within each landscape, 2-3 fields were selected and 5-10 geo-referenced samples per field were collected from 0-30 cm and 30-60 cm depths. Soil food web structure varied by temperature and/or soil group in the top 30 cm. Principal component analyses of the measured parameters showed distinct correlation patterns among soil groups, by soil group within a temperature zone, by depth of sampling and/or landscape. Overall, the data suggest that the soil groups may have to be treated differently within or across temperature zones when considering soil health management strategies.

HOST-RESPONSE OF *PRUNUS* ROOTSTOCKS TO AGGRESSIVE *MELOIDOGYNE* SPP. ISOLATES SELECTED FROM THE CENTRAL VALLEY OF CHILE. **Meza, P.¹, L. Rojas¹, and D. Esmenjaud².** ¹Instituto de Investigaciones Agropecuarias, INIA, Centro Regional La Platina. Av. Santa Rosa N°11610, La Pintana, Santiago, Región Metropolitana-Chile. ²ISA, INRA, Université de Nice-Sophia Antipolis, CNRS, 06900 Sophia Antipolis, France. Corresponding author email: pablo.meza@inia.cl. Proyecto Fondecyt N°11121209.

In Chile, stone fruits (*Prunus* spp.) are mainly produced in four Chilean regions of the Central Valley (latitude 30°S-37°S), where they account for 80% of the national fruit production area. In these regions, root-knot nematodes (RKN) *Meloidogyne* spp. have a high economical impact and *M. ethiopica* is the prevalent species on many crops. The use of resistant rootstocks is one of the most economical and environmentally sound methods for managing RKN infestations. The aim of this study was to evaluate the host response of ten *Prunus* rootstocks: Pomona, Nemaguard, Mariana2624, CAB-6p, Gisela-6, Piku-1, Piku-4, Rootpac-90, Rootpac-20 and Rootpac-R, against six aggressive *Meloidogyne* isolates. These isolates, three from each *M. ethiopica* and *M. javanica*, had been rated in a previous study as the most aggressive out of 20 RKN isolates sampled in the Central Valley. Ten replicates of each rootstock were used and inoculated with 10,000 individuals (J2+eggs) containing an equal proportion of the six isolates. The infested rootstocks were grown in pots under greenhouse conditions for five months and then harvested. Numbers of galls and nematodes were counted and reproduction data were analyzed separately using a one-way analysis of variance. The host response of *Prunus* rootstocks was rated as follows: I = immune (absence of root galls; no nematodes in roots); HR = highly resistant (very low galling and final/initial numbers < 0.10); R = resistant (low galling; 0.10 < final/initial numbers < 1); and S = susceptible (abundant galling; final/initial numbers > 1). The rootstock Pomona was susceptible. Nemaguard and Rootpac-90 were resistant and highly resistant, respectively, whilst Mariana2624, CAB-6p, Gisela-6, Piku-1, Piku-4, Rootpac-90, Rootpac-20 and Rootpac-R were immune.

VERMICOMPOST TEA MEDIATED PLANT RESISTANCE AGAINST ROOT-KNOT NEMATODES, *MELOIDOGYNE* SPP. **Mishra, S., B.S. Sipes, M. Tian, and K.-H. Wang.** Department of Plant and Environmental Protection Sciences, 3050 Maile Way, Honolulu, HI 96822.

Vermicompost tea (VCT) is a water extract of vermicompost produced by mesophilic decomposition of organic matter through interactions between earthworms and microorganisms. VCT has been reported to suppress root-knot nematodes (*Meloidogyne* spp.) in greenhouse conditions. However, the effects of vermicompost tea on suppressing root-knot nematodes (RKN) are inconsistent. Laboratory trials verified that VCT prepared from uncured vermicompost using plant waste as feed stock suppressed root penetration and egg hatch of *M. incognita* more effectively than cured vermicompost. It is hypothesized that beneficial microorganisms present in VCT played a role in the suppression of RKN. Two field trials were conducted to

determine drenching frequency of VCT to suppress RKN in a cucumber (*Cucumis sativus*) agroecosystem. Cucumber plants were drenched at 1-, 2- or 4-week intervals and compared to a water control. In both trials, drenching VCT at 1-week intervals suppressed ($P \leq 0.05$) RKN but not reniform (*Rotylenchulus reniformis*) nematodes. VCT could induce host-plant resistance, consequently two split-root experiments were conducted in the greenhouse. Cucumber root penetration by of *M. incognita* was reduced by VCT drenching. Quantitative real time PCR was used to detect the expression of the defense related genes *CHIT-1* and *PAL-1* encoding chitinase and phenylalanine ammonia-lyase, respectively. Cucumbers were drenched with VCT from uncured vermicompost 2 days prior to *M. incognita* inoculation. Gene expression levels in leaves were determined at 0 hr, 1, 2, 5 and 8 days post nematode inoculation. Plants drenched with VCT showed an increased expression of *CHIT-1* at 2 days after *M. incognita* inoculation, and increased expression of *PAL-1* at 0, 1, 2 and 8 days after *M. incognita* inoculation. Additional assays were conducted to study the expression of β -1,3-Glucanase, *LOX-1* and *PR-1* encoding glucanase, lipoxygenase, and pathogenesis-related protein 1 respectively. These experiments suggested that VCT prepared from uncured vermicompost has the potential to induce host-plant resistance against RKN. Although this induction was short in duration, drenching VCT at a weekly interval provided significant and consistent suppression of root-knot nematodes throughout a cucumber crop. Thus, use of VCT prepared from an uncured vermicompost is a viable post-plant nematode management tool against RKN. Future work could include applying VCT through fertigation and modifying feed stock of vermicompost to achieve reniform nematode suppression by VCT.

PHLOROGLUCINOL (1,3,5-TRIHYDROXYBENZENE) ENHANCED THE AMELIORATES STRESS RESISTANCE AND REDUCED β -AMYLOID TOXICITY IN *CAENORHABDITIS ELEGANS*. Mohankumar, A¹., G. Shanmugam¹, P. Sundararaj¹, S.L. Hafez² and Nivitha Sundararaj³. ¹Unit of Nematology, Department of Zoology, Bharathiar University, Coimbatore; ²U of I Parma REC, 29603 U of I Lane, Parma, ID 83660, USA ³Kumaraguru College of Technology, Coimbatore, India.

Phloroglucinol (PG), found in brown algae (Phaeophyceae), is a monomeric unit of phlorotannins with antioxidant, anti-inflammatory, anti-diabetic, antimicrobial, anti-allergic and anti-viral properties. An attempt has been made to study the effect of PG on the lifespan, health, β -Amyloid toxicity and oxidative stress tolerance on the nematode model *Caenorhabditis elegans*. Various concentrations of PG viz, 10 μ M, 50 μ M, 100 μ M, 200 μ M, 400 μ M, 800 μ M, 1000 μ M were prepared using DMSO (final concentration to be maintained at 0.2%) and treated on *C. elegans*. For lifespan assay, *C. elegans* eggs were transferred to the NGM plates previously treated with or without PG and *E. coli* OP50 and maintained at 20 °C. Synchronization was performed at L4 larvae stage, and young L4 larvae were transferred to fresh plates with different PG concentrations (40 worms per plate with a total of 3 plates per treatment). Worms were observed daily for survival and transferred to fresh treatment plates until the last worms dead. At 400 μ M PG, the mean lifespan of wild type N2 and mev-1 *C. elegans* increased while there was no increase in longevity of daf-16 mutant worms. Exposure of wild *C. elegans* to PG at 400 μ M did not induce any changes in body length, morphology, brood size, locomotion or mortality while there was a significant decrease in lipofuscin accumulation. Pre-treated *C. elegans* with PG suppressed the damage due to heat stress and oxidative stress induced by an ROS generator, Juglone (500 μ M). PG treated worms had increased fluorescence intensity of HSP-16.2 and SOD-3 proteins compared to untreated control strains of CL2070 and CF1553 respectively. PG delayed the amyloid- β induced paralysis up to 25.4% compared to control worms in transgenic *C. elegans* strain CL4176 expressing human Amyloid β ₁₋₄₂. Thus, the results suggest that the insulin/IGF-1 signalling and their downstream and mitochondrial respiratory chain pathways are involved in the mechanism of life extension and stress tolerance mediated by PG.

FIRST REPPORT AND ADDITIONAL INFORMATION OF *MELOIDOGYNE KONAENSIS* PARASITIZING DIFFERENT CROPS IN BRAZIL. Monteiro, J.¹, J.E. Cares¹, A.C.M.M. Gomes², V.R. Correa², M. F. A. Santos², M.D.G. Carneiro², G. Gomez², C.D.G. Santos³, and P. Castagnone-Sereno⁴, and R.M.D.G. Carneiro². ¹Departamento de Fitopatologia, Universidade de Brasília, Brasília DF 70910-900, Brazil, ²EMBRAPA- Recursos Genéticos e Biotecnologia, CP.02372 Brasília DF 70849-970, Brazil, ³Departamento de Fitopatologia, Universidade Federal do Ceará, Fortaleza CE 60020-181, ⁴INRA, Univ. Nice Sophia Antipolis, CNRS, UMR 1355-7254, Institut Sophia Agrobiotech, 06900 Sophia Antipolis, France.

In a survey for *Meloidogyne* spp. in different crops from eleven regions at Ceará State, Brazil using esterase isozyme electrophoresis as a specific identification method, four atypical populations were characterized from cabbage, papaya, noni and canapum plants, which showed an esterase profile different from those previously detected in Brazil. Morphological studies showed typical characteristics of the specie *Meloidogyne konaensis*. Perineal patterns of females were variable, similar to *M. arenaria* and *M. incognita*, stylet length 14-20 μ m, the knobs gradually merging with the shaft and the dorsal esophageal gland orifice (DEGO) ranging from 4-7 μ m, are some characteristics of females. Although males are not frequently found, the stylet morphology provides the most useful source of diagnostic character for the specie, with 6-12 large projections protruding from the shaft. The esterase pattern K3 is unique and species-specific with three major bands Rm 1.0, 1.17, 1.27 and a secondary band Rm 1.10. Some confusion about the true identity of this species was clarified in this study including the differentiation from *M. paranaensis*. A species-specific SCAR marker developed for *M. paranaensis* was

tested and no amplification products were observed. In Neighbour-Joining analyses of ITS and D2-D3 rRNA sequences, *M. konaensis* from Brazil appeared clearly separated from *M. paranaensis*. Pathological tests indicated that coffee is not a host of *M. konaensis* as previously reported in the original description of this species.

FIRST REPORT OF ENDOTOKIA MATRICIDA IN *MELOIDOGYNE HAPLA*: A STUDY CASE. **Monteiro, T.S.A.¹, J.A. Brito², S.J.S. Vau⁴, W. Yuan², J.A. LaMondia³, and D.W. Dickson⁴.** ¹Plant Path. Dept., Univ. of Vicosa, Vicosa, MG, 36570-900, Brazil, ²Div. of Plant Industry, FDACS, Gainesville, FL 32614-7100; ³Connecticut Agri. Exp. Sta., Windsor, CT 06095, ⁴Entomology and Nematology Dept., Univ. of Florida, Gainesville, FL 32611-0620.

Endotokia matricida is a phenomenon known to occur when embryogenesis and egg hatching takes place within the nematode uterus. This phenomenon is known to occur within the Rhabdita, but it is very uncommon among plant-parasitic nematodes. This phenomenon was observed in a population of *Meloidogyne hapla*, originally collected in Connecticut, USA and reared on tomato 'Rutgers' maintained at 24°C for 60 days in a growth room. Out of 974 females examined, only 14 showed this phenomenon or 1.44%. Eggs at different stages of development, including first-stage juveniles within the egg and second-stage juveniles (J2) were observed inside of the female body cavity. The highest number of J2 and eggs observed per female showing endotokia matricida was 57 and 350, respectively. However, the average number of J2/female was 13, whereas the average number of eggs/female was 90. These findings suggest that this phenomenon is not common in the population of *M. hapla* used in this study. To the best of our knowledge this is the first report of endotokia matricida in *M. hapla*.

VIRAL INFECTION IN NEMATOPHAGOUS FUNGUS *POCHONIA CHLAMYDOSPORIA*. **Monteiro, T.S.A.¹, A.P. Oliveira², A.S. Xavier¹, P. Alfenas-Zerbini² and L.G. Freitas¹.** ¹Plant Pathology Dept., University of Viçosa, Viçosa, MG, 36570-900, Brazil, ²Microbiology Dept., University of Viçosa, Viçosa, MG, 36570-900, Brazil.

Pochonia chlamydosporia is a nematophagous fungus which has been widely studied because of its ability to reduce nematode populations and to promote plant growth. The differences in the virulence against nematodes, common among isolates of *P. chlamydosporia*, may be due to the genetic constitution or to the interactions with other microorganisms or the environment. The mycoviruses cause cryptic infections and may be responsible for alterations in the fungus aggressiveness (hypo/hypervirulence). The *P. chlamydosporia* isolate PCM4 was the only infected out of 12 investigated. The virus found has a standard complex dsRNAs, multisegmented or a mixed infection. In a greenhouse experiment, this strain failed to reduce the population of the root-knot nematode, *Meloidogyne javanica*. These findings infer the importance of indexing when screening isolates for biological control of nematodes. Future trials using a virus-free line of PCM4 would help to understand if the low nematode antagonism in this isolate is the result of the viral infection or not. This is the first report of a mycovirus infecting *P. chlamydosporia*.

HOW LOCAL SCALE VARIATION INFLUENCES NEMATODE COMMUNITY STRUCTURE IN THE FYNBOS MEDITERRANEAN HEATHLAND OF SOUTH AFRICA. **Moroenyane, I.¹, K. Dong², S.B.M. Chimphango³, D. Singh⁴, and J.M. Adams².** ¹Institut National de la Recherche Scientifique, Centre INRS-Institut Armand-Frappier, 531 Boulevard de Prairies, Laval, Québec, H7V 1B7, ²Department of Biological Sciences, College of Natural Sciences, Seoul National University, Seoul 151-742, South Korea, ³Department of Biological Sciences, University of Cape Town, Cape Town, South Africa, 7700, ⁴Environmental Genomics Division, CSIR-NEERI, Nehru Marg, Nagpur (MH), 440020, India.

The Fynbos biome of South Africa is renowned for its high levels of plant diversity, endemism, and heterogeneous soils. Studies have elucidated the broad taxonomic classification and diversity patterns of soil nematodes in Fynbos. However, majority of these studies have only looked at the diversity of plant feeding nematode, and none have compared the community of free-living nematodes from different Fynbos types. We used a novel metagenetic approach to investigate variation in nematode community structure in the Fynbos vegetation. We compared 23 samples of soil nematode communities from five different Fynbos vegetation types. Nematode DNA was 454-pyrosequenced for the 18S rRNA gene. Here we show that soils from Fynbos sites (Alluvial, Sand, Limestone, Shale, and Sandstone) have distinct properties and how these influence the nematode community structure and diversity. Previous studies of free-living nematode, revealed great diversity and differentiation of nematode community structure along an environmental gradient. We found that the diversity (Shannon and Simpson index) was overall significant, but there was no difference between sites and there was no environmental gradient that seemed to delimit this diversity. Similarly, the relative abundance of dominant nematode families did not vary across sites, with the exception of *Tylenchidae*. The relative abundance of feeding guilds also did not vary across sites; interestingly, only plant feeding nematodes significantly varied and were negatively correlated with NH₄. Furthermore, the nematode community based on Bray Curtis distance did not cluster by Fynbos vegetation type and was significantly influenced by potassium (K) and sulphate (SO₄). However, the phylogenetic signal detected that closely related taxa in Fynbos tend to co-occur more often than expected by chance. Unifrac analyses also did not cluster by vegetation type, but was influenced by geographical distance. It seems that in the Fynbos there has been very little phylogenetic divergence (ecological and genetic drift) of nematode lineages. Furthermore, at local scale the ses.NTI (nearest taxon index) was significantly higher than null expectations, indicating that co-occurrence of related nematode lineages is determined by the differences in environmental

conditions across the sites. We hypothesize that in the Fynbos there is niche overlap between closely related nematodes, and nematode speciation tends to occur conservatively into closely related niches. We further propose that the phylogenetic community structure at the local scale is assembled by deterministic (rather than neutral) processes.

CAENORHABDITIS ELEGANS RESISTANCE TO THE BACTERIAL PARASITE *SERRATIA MARCESCENS*: EVIDENCE FROM EXPERIMENTAL EVOLUTION AND NATURAL POPULATIONS. **Morran, L. ¹, M.J. Penley¹, P.S. White¹, and A. Paulk¹.** ¹Department of Biology, Emory University, Atlanta, GA 30622.

Selection imposed by antagonistic coevolution can dictate the evolutionary trajectories of populations and is one of the primary sources of evolutionary change in nature. Host populations have evolved a diverse array of mechanisms to facilitate resistance against parasite infection. We set out to characterize the mechanisms of resistance employed by the host nematode, *Caenorhabditis elegans*, in the presence of the bacterial parasite, *Serratia marcescens*. We previously experimentally evolved host populations of the nematode with the parasite and subsequently observed an increase in host fitness in the presence of the parasite. Here, our goal was to determine the mechanism of resistance evolved by the host populations in response to selective pressure imposed by the parasites. Overall, we found that adult *C. elegans* hosts rapidly evolved changes in food preference, ultimately avoiding exposure to *S. marcescens* as a result. Ancestral adults preferred *S. marcescens* relative to non-parasitic *Escherichia coli*, whereas evolved adults exhibited a strong preference for *E. coli*. Our results demonstrate that parasite avoidance is at least one form of resistance that can be employed by *C. elegans*, and that avoidance of *S. marcescens* likely contributed to the increased fitness exhibited by the evolved hosts. Next, our goal was to determine if *C. elegans* natural isolates exhibited parasite avoidance in the presence of *S. marcescens*. Corroborating results from previous studies, we found that *C. elegans* adults derived from natural isolates generally exhibited a preference for *S. marcescens* relative to *E. coli*, rather than avoidance, despite the fact that these species encounter one another in nature. However, contrary to previous studies, we also assayed *C. elegans* dauer larvae for bacterial food source preference because the dauer life stage is the dispersal life stage in nature, much like the adult life stage was the dispersal phase during experimental evolution. We found that dauer larvae of multiple *C. elegans* natural isolate strains exhibited a preference for *E. coli* over *S. marcescens*, indicating that parasite avoidance may be a widespread phenomenon in *C. elegans*. Taken together, these results demonstrate that *C. elegans* can utilize parasite avoidance as a mechanism of resistance and that selection favoring the evolution of avoidance behavior may be particularly strong during dispersal. Further, this work adds to a growing body of evidence rapidly establishing the evolution of host behavior as an integral factor in disease evolution.

POTENTIAL OF *HETERORHABDITIS INDICA* TO CONTROL *CYLAS FORMICARIUS* IN FIELD CULLED SWEET POTATO ROOTS. **Myers, R., G.T. McQuate, C.D. Sylva, and C.L. Mello.** USDA ARS Daniel K. Inouye U.S. Pacific Basin Agricultural Research Center, 64 Nowelo St, Hilo, HI 96720.

Sweet potato weevil, *Cylas formicarius*, is one of the most destructive insect pests of sweet potato in Hawaii. The larvae feed and tunnel inside the root causing malformation and a bitter taste that makes the product unmarketable. During harvest, farmers leave off-grade roots in the field which serve as inoculum and infect the next planting. Sanitation by removing roots and vines is the current control measure but rarely practiced due to high labor costs. An indigenous entomopathogenic nematode, *Heterorhabditis indica*, was shown to infect and kill *C. formicarius* in laboratory studies. In petri dish assays, 90% of larvae and nymphs were killed in two days when inoculated with 1 – 25 infective juveniles (IJs). In tub bioassays, tuberous roots infected with *C. formicarius* were partially buried in sand and inoculated with *H. indica*. After one month, 89% and 100% of larvae and pupae from dissected root slices were morbid after inoculation with 1,000 and 10,000 IJs respectively. The remaining root pieces were placed on modified white traps and held for 25 days. An average of 60,000 nematodes was recovered from each tuberous root. Spraying these biological control agents on harvested fields has potential as a sanitation measure for reducing sweet potato weevil populations between plantings.

NEW NON-FUMIGANT CONTACT NEMATICIDE, NIMITZ[®], UPDATE ON NEW COMING LABEL. **Navia Gine, P.A.** 925 Country Wood Ct. Wellington, FL 33414.

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

currently registered in the 26 primary vegetable producing states and Puerto Rico. Registration on new crops already submitted to the EPA, new label pending. Multiple country registrations are pending.

NOVEL COWPEA ACCESSION PROVIDES A BROAD-BASED RESISTANCE FOR BREEDING ROOT-KNOT NEMATODE RESISTANT CULTIVARS. Ndeve, A.¹, W.C. Matthews¹, J.R.P. Santos¹, B.L. Huynh¹, T.J. Close² and P.A. Roberts¹. ¹University of California Riverside, Dept. Nematology, Riverside CA 92521, USA; ²University of California Riverside, Dept. Botany and Plant Sciences, Riverside CA 92521.

Root-knot nematodes (RKN), *Meloidogyne incognita* and *M. javanica* cause substantial damage on cowpea and account for low yields in infested areas. The gene *Rk* is predominantly used to manage RKN in cowpea cropping systems; however, it is ineffective *Rk*-virulent populations. Through genetic studies, the value of cowpea accession FN-2-9-04 for breeding cowpea cultivars with broad-based RKN resistance was determined. To examine inheritance and to determine allelic relationship between the gene *Rk* and resistance factors in FN-2-9-04, susceptible genotypes (no-*Rk*) and a commercial cultivar carrying gene *Rk* were crossed with FN-2-9-04, respectively. F₁, F₂ populations and F₃ families were phenotyped for root galling (GI) and egg mass production (EM) responses to *M. javanica* and avirulent *M. incognita*. Plants carrying no-*Rk*, *Rk* and stacked *Rk* genes (*Rk/Rk2*, *Rk/Rk2/gg* and *Rk/rk3*) were used as controls. Analysis of segregation indicated that all F₁ plants were resistant based on GI and EM, while the segregation in the F₂ and F₃ (no-*Rk* x FN-2-9-04) fit a 13:3 ratio ($P < 0.05$) suggesting a response under control by two genes, a dominant and a recessive. The F₂ population (*Rk* x FN-2-9-04) did not segregate for GI under avirulent *M. incognita* indicating that the FN-2-9-04 might carry a RKN resistance gene equivalent to or linked to the locus *Rk*. Conversely, this population segregated for EM in a 13:3 ratio ($P < 0.05$) under *M. javanica* infection. These data, suggest that a recessive gene is required for the broad-based resistance which is effective against RKN. The genomic architecture of resistance factors in the NA will be determined through genetic mapping with SNP markers to confirm their value for breeding RKN resistant cowpea cultivars.

EFFECTS OF LAND-USE INTENSITY ON THE COMMUNITY COMPOSITION OF NEMATODES FROM CAO BANG PROVINCE IN VIETNAM. Nguyen, T.A.D.^{1,2,3}, J. Abolafia³, R. Peña-Santiago³ and M. Bonkowski¹. ¹Institute for Zoology, Department of Terrestrial Ecology, University of Cologne, Zùlpicher StraÙe 47 b, D-50674 Kùln, Germany. ²Institute of Ecology and Biological Resources (IEBR), Vietnam Academy of Sciences and Technology, 18 Hoang Quoc Viet, Hanoi, Vietnam. ³Department of Animal Biology, Plant Biology and Ecology, University of Jaén, 23071 Jaén, Spain.

Increasing land use intensity has likely strong impacts on soil biodiversity and fertility in the tropics. Nematodes are known to respond rapidly to soil disturbance and changing resources. Therefore, the functional composition of the nematode community offers a reliable measure for the biological assessment of the quality and functioning of tropical soils. In Cao Bang we investigated the diversity of soil nematode communities at 24 sites. To analyze how local land management interferes with species richness, the soil samples were taken along gradients of increasing land use intensity (primary rain forest, secondary forest, slash and burn agriculture/casava, and intensive agriculture), to investigate changes in nematode community composition, soil carbon storage and nutrient cycling. Although nematode community composition differed between regions, land use intensity had distinct impacts on the functional composition and food web structure of nematodes. Indicators of these changes, such as the Maturity Index, Channel Index or Plant Parasitic Index were correlated to changes in soil carbon and nutrient levels. The tropical forests of Cao Bang are known for their high diversity of plants and vertebrates, because still a high proportion of pristine primary forest is found here. Interestingly, the high Maturity Index of the nematode communities of the primary forests in Cao Bang exactly reflected these findings for the soil communities, but it also revealed a strong gradient of increasing disturbance of sites with increasing land use intensity. In particular the Plant Parasitic Index reflected a shift towards root-endoparasitic nematode taxa with increasing agricultural land use, and the Channel Index showed a strong shift towards the bacteria-based decomposer channel in agricultural sites compared to a more stable energy transfer through the 'slow' fungal decomposition channel in forest habitats.

DEMONSTRATING THE IMPORTANCE OF VERTICAL MANAGEMENT ZONES FOR STING NEMATODE CONTROL AND CROP RESPONSE IN FLORIDA STRAWBERRY. Noling, J.¹, G.E. Vallad² and N. Boyd². ¹University of Florida, IFAS, Citrus Research & Education Center, Lake Alfred, FL 33850, ²Gulf Coast Research and Education Center, Wimauma, FL 33598.

Since the phase-out of methyl bromide (MBr) in 2015, a reliable and consistent soilborne pest and disease control program has not been fully established, with incidence and severity of nematode problem fields increasing. Unlike MBr, the alternative fumigants, with current application methods, are unable to distribute vertically below a traffic pan, located just below the level of the raised bed. The presence of the subsurface traffic pan (a dense, highly compacted soil layer), has been shown to restrict hydraulic conductivity, permeability to fumigant gases, and crop root penetration into deeper soil. We hypothesized that treating these different vertical zones, those above and below the traffic pan, is critical for nematode control and for sustaining crop production. During 2015, field studies were initiated to demonstrate the importance of deep fumigant placement and need for considering sting nematode control as a composite of vertical management zones. Two different tractor mounted hydraulic soil sampling probes, extracting either 3.8 or 10 cm soil cores 122 cm deep were used to

characterize the spatial distribution of plant pathogenic nematodes in commercial strawberry fields employing a variety of crop and nematode management practices. The results from these field samplings have repeatedly shown that sting and or root-knot nematode can inhabit very deep soil profiles, below the traffic pan and well below the depths to which any of the current shank or drip applied fumigants diffuse. The depths to which nematodes reside are also below depths where plant roots are found and well below depths that are typically ever sampled for nematodes. To target deep soil profiles, new fumigant application systems were developed to make deep shank or deep drip fumigant applications to a depth of 40 cm (16"). Three nematicide treatments including deep shank and or drip applications of 1,3-dichloropropene (Telone™; 140-168 L/ha), with or without a grower standard treatment to the plant bed, were evaluated in 10 commercial strawberry fields in Dover, FL. Each treatment was replicated at least 6 times. Broadcast or in-row (strip) deep shank 1,3-D were evaluated in separate trials. Soil population densities of sting and or root-knot nematode were significantly lower at seasons end with either deep drip or deep shank 1,3-D applications compared to the grower standard. When the grower standard was applied to the raised plant bed, supplemental deep shank treatments of 1,3-D increased strawberry yields by as much as 9 to 29% in many of the fields. Even without significant nematode pressure in the field, crop health, vigor, and appearance were always enhanced, and yields, though not significant in these instances, were always numerically higher in the supplemental deep shank or deep drip 1,3-D treatments compared to the grower standard. We believe a primary cause of inconsistent nematode control using MBr alternatives has been identified, and that supplement fumigant applications, which consider the importance of vertical management zones, will be required to manage nematode pests in Florida strawberry.

USE OF MAJESTENE AS A CROP RESUE TREATMENT IN FLORIDA STRAWBERRY FOR CONTROL OF THE STING NEMATODE, *BELONOLAIMUS LONGICAUDATUS*. **Noling, J.** University of Florida, IFAS, Citrus Research & Education Center, Lake Alfred, FL 33850.

In Florida, the Sting nematode (*Belonolaimus longicaudatus*) infests an estimated 40% of strawberry acreage. Any loss of nematode control from preplant fumigant treatment typically results in a higher incidence of plant stunting in the field. In most years, plant stunting is expressed relatively early in the season, with ultimate size and yield functionally determined by nematode concentration x time products over the season. Florida strawberry growers have longed for a post plant nematicide to control Sting nematode and increase strawberry crop productivity. Majestene™, a new bacterial based bionematicide was evaluated as a post plant crop rescue treatment against the Sting nematode in six commercial strawberry fields in Dover, FL. Fields were selected based on their diversity and similarity of nematode induced stunting of plants in rows adjacent to untreated check rows. Treatments consisted of either one or two mid-season Majestene applications for a total rate of 9.4 to 40 L/ha. Each treatment was replicated six times at each field site and compared with an adjacent untreated plant row. Field sizes ranged from 2 to 10 hectares. All applications used the drip irrigation system, utilizing either 1 or 2 drip lines per bed, to deliver Majestene over a 30 to 64 minute injection period followed by a 20 to 30 minute irrigation flush. Nominal flow rates for individual drip emitters were 0.6 and 1.4 L/hr at 10 psi for one and two drip tapes respectively. Soil samples for Sting nematode population density determinations were taken prior to Majestene chemigation treatment and 8 weeks post treatment. Strawberry plant canopy diameters were measured prior to Majestene treatment in each infested field. The average of two separate bidirectional measurements was permanently recorded to the plastic mulch covering the raised strawberry plant bed so as to provide record of initial canopy size prior to treatment. Plant canopy measurements were reacquired from the same premeasured plants 6 to 8 weeks after Majestene treatment in each field. Positive or negative changes in canopy diameter were compiled from 75 to 90 individual plants from each treatment and in each field. In general, Majestene provided no apparent benefit to Sting nematode control at any of the six field sites. No significant improvement to plant growth and canopy size associated with Majestene treatment was observed at any field site. In general, the smaller the initial plant size the more negative the change in plant growth, suggesting the difficulty in rescuing a severely stunted plant. Plant canopy size did increase in one field, independent of Majestene application, following repeated foliar applications (1.12 kg/ha) of 20-20-20 NPK fertilizer. This work suggests the need for additional, more defining research to quantify the dose response relationship for different nematode species, optimal concentration and injection period within the irrigation stream, and to clarify appropriate times within the cropping season in which efficacy and plant growth benefit to infected plants can be effectively achieved.

LOCAL AND SYSTEMIC RESPONSES OF SUSCEPTIBLE AND RESISTANT TOMATO (*SOLANUM LYCOPERSICUM*) GENOTYPES FROM GHANA TO ROOT-KNOT NEMATODE (*MELOIDOGYNE INCOGNITA*). **Nyaku, S.T.^{1,3} and U. Paszkowski^{2,3}**. ¹Department of Crop Science, College of Basic and Applied Sciences, University of Ghana, P.O. Box LG 44, Legon-Accra, Ghana, ²Department of Plant Sciences, University of Cambridge, Downing Street, Cambridge, CB2 3EA, ³Cambridge-Africa Programme, Department of Pathology, University of Cambridge, Tennis Court Road, Cambridge CB2 1QY.

Tomato (*Solanum lycopersicum* L.) is a major part of the diet in Ghana. However, tomato production in Ghana is hampered by biotic agents such as *Meloidogyne incognita*, a widely distributed pathogen across the tomato-cultivating regions of the world. Elucidation of the molecular mechanisms underlying the plant defense responses against *M. incognita* is of utmost importance for the development of alternatives to environmentally toxic chemicals. Resistant tomato genotypes produce an incompatible interaction with *M. incognita* which results in a hypersensitive response (HR), generation of ion fluxes, and

reactive oxygen species. Ethylene (ET), Salicylic acid (SA), and jasmonic acid (JA) signaling pathways are involved in inducing defense responses against these pathogens. Our objective is to investigate the local and systemic responses of susceptible and resistant tomato varieties to *M. incognita* invasion. Using a split root technique, half of the root system was inoculated with juveniles of *M. incognita*. Quantitative PCR analysis was used to comparatively determine the expression of ET, SA, and JA-regulated marker genes.

EFFECTIVENESS OF *ACALYPHA WILKESIANA* IN MANAGING PLANT PARASITIC NEMATODES AND BANANA WEEVIL ON PLANTAIN AND ENHANCING PLANTATION LONGEVITY. **Olaniyi, M.O.¹, A.O. Oso², and O. Alabi³.** ¹School of Science and Technology, National Open University of Nigeria, 14/16 Ahmadu Bello Way, Victoria Island, Lagos, Nigeria, ²Dept. of Crop, Soil and Environmental Sciences, Ekiti State University, Ado-Ekiti, Nigeria, ³Cassava Breeding Program, International Institute of Tropical Agriculture, Oyo road, Idi-Ose, Ibadan, Nigeria.

Damage by pests like plant parasitic nematodes and banana weevil (*Cosmopolites sordidus*), and other confounding abiotic factors often resulted in abandonment of plantain (*Musa* spp, AAB-subgroup) plantations after two to three production cycles due to yield decline. The efficacy of crude water extract of *Acalypha wilkesiana* at managing plant parasitic nematodes and banana weevils on plantain was investigated over a period of three and half years; from screen house to the field across two production cycles. In a pilot screen house study which lasted seven weeks, no beneficial ($P \leq 0.05$) effect of crude water extract of *A. wilkesiana* was obtained on vegetative growth and root damage over simply paring the corm of the sucker planting material. However, a short-term (16 weeks) field trial to determine optimum duration of exposure to the extract indicated that pre-plant dipping of pared sucker planting materials in crude water extract for 15 min could reduce root damage as well as nematode densities. Consequently, the effect of 15 min dip of sucker planting material in crude water extract on plantain yield and plantation longevity was investigated across two production cycles of three years: mother crop and first ratoon. There was no significant ($P \leq 0.05$) reduction in the densities of *Helicotylenchus multicinctus*, *Meloidogyne* spp and *Pratylenchus coffeae* across the treatments although lower numbers were recovered from plots established with pared, extract-treated plants. Fifteen months after planting in the field, non-pared suckers produced etiolated plants with fewer functional leaves. When suckers were pared before planting, there were fewer weevils in the plots. Generally, paring supported higher yield in both mother and ratoon crops. Whereas there was decline in yield in the second cycle for other treatments, extract-treated, pared suckers had more than 15% yield increase over the untreated pared suckers in the second cycle and more than 47% yield advantage over the non-pared planting materials. *Acalypha wilkesiana* would be useful in checkmating plantain yield decline and enhancing plantation longevity. Conclusively, short-term study is not adequate to extrapolate a long-term effect of treatments under field conditions for plantain, while certain vegetative response to treatment in plantain may not necessarily elicit similar trend in yield response.

ENDOSIMBIOTIC BACTERIA FROM THE GENUS *CANDIDATUS XIPHINEMABACTER* AND THEIR CO-EVOLUTION WITH *XIPHINEMA AMERICANUM* GROUP SPECIES (NEMATODA: DORYLAIMIDA). **Orlando, V.¹ and S.A. Subbotin².** ¹Via Giacomo Puccini 10, 90017 Santa Flavia, Italy, ²Plant Pest Diagnostic Center, California Department of Food and Agriculture, 3294 Meadowview Road, Sacramento, CA 95832-1448, USA.

The *Xiphinema americanum* group contains over two-dozen different nematode species, several of them are economically important because they are vectors of nepoviruses. Three bacterial endosymbiont species, *Candidatus Xiphinematobacter americani*, *Ca. X. rivesi* and *Ca. X. brevicolli* have been described from three nematode species, *X. americanum*, *X. rivesi* and *X. brevicolle*, respectively. It has been hypothesised a long-term co-evolution between *Xiphinema americanum* group species and their symbionts. Although, recently published studies revealed a high genetic diversity between endosymbionts, their phylogenetic relationships to their hosts have not been studied properly. We sequenced the partial bacterial 16S rRNA gene of 55 nematode samples from seven species of the *X. americanum* group and compared them with those published in the GenBank. Using the percent identity of the 16S rRNA gene as the cutoff for species delimitation, we distinguished 17 species of bacterial symbionts from the genus *Ca. Xiphinematobacter*: *Ca. X. rivesi*, *Ca. X. americani*, *Ca. X. brevicolli* and fourteen undescribed species, five of which are reported for the first time. Each nematode species carries a unique species or group of species of *Ca. Xiphinematobacter*. The *Xiphinema brevicolle* complex was associated with five bacterial species, *X. rivesi* with three species, *X. californicum* with two species and several other *Xiphinema* species, including *X. simile* and *X. browni*, with one species. Comparison of nematode *coxI* and bacterial 16S rRNA gene phylogenies, using both global fit and event based methods, revealed significant congruence between nematode and bacterial trees and high numbers of co-speciation events in nematode-bacteria associations.

GENOMIC AND PROTEOMIC APPROACHES TO INVESTIGATE SECONDARY METABOLITE PRODUCTION OF *PHOTORHABDUS L. SONORENSIS* (ENTEROBACTERIACEAE) IN ITS MUTUALISTIC AND PATHOGENIC STAGES. **Orozco, R., A. Castagnola and S.P. Stock.** Department of Entomology, University of Arizona, 1140 E. South Campus Dr., Tucson, AZ 85721-0036, United States.

Gram-negative *Photorhabdus* bacteria produce a diverse array of small molecules that play key biological roles in regulating their dual lifestyle: mutualists to *Heterorhabditis* nematodes, and pathogens of insects. In particular, secondary metabolites these bacteria produce in the insect cadaver are known to play a critical role in the maintenance of a

monoaxenic infection and also in preventing contamination from soil microbes and/or predation by other arthropods. The genomes of several *Photorhabdus* spp. have revealed the presence of numerous gene clusters that encode enzymes for secondary metabolite (SM) biosynthesis. These biosynthetic gene clusters are known to comprise more than 6% of their genomes. Furthermore, genome mining and characterization of biosynthetic pathways, has uncovered the richness of these compounds which are predicted to vary across different *Photorhabdus* species and strains. In this respect, we are currently investigating *Photorhabdus l. sonorensis*, the bacterial symbiont of an Arizona-native nematode, *Heterorhabditis sonorensis*. Specifically, we are interested in characterizing gene clusters involved in production of symbiosis factors, as well as SM enzymes, and other proteins produced during the pathogenic stage of this bacterium. A genomic approach coupled with a proteomic analysis has revealed the presence of many gene clusters that are predicted to encode novel molecules. Results from this study will be presented and discussed.

A METAGENETIC TOOL TO STUDY NEMATODE AND *PASTEURIA* POPULATION DYNAMICS. Orr, J.^{1, 2}, P. Cock¹, K.G. Davies², and V.C. Blok¹. ¹Cell and Molecular Sciences, The James Hutton Institute, Invergowrie, Dundee DD2 5DA, ²Department of Biological and Environmental Sciences, University of Hertfordshire, Hatfield, AL10 9AB.

Pasteuria spp. are spore forming bacterial microparasites which act as natural antagonists of many of the most economically devastating plant parasitic nematodes (PPNs). As obligate and fastidious parasites, *Pasteuria* spp. may contribute to the development of highly specific suppression of PPNs in soil via two key mechanisms: inhibition of root invasion; and sterilisation. At present the tools available to study the dynamics of these interactions rely largely on the extraction and direct observation of spore encumbered or spore filled nematode bodies. With these methods it is impossible to direct the recovery of novel or understudied pathotypes of *Pasteuria* spp. without the benefit of existing data indicative of specific PPN suppression. First, we have demonstrated that PCR based detection methods can be used to direct the detection and recovery of *Pasteuria* spp. from environmental samples. Of 144 nematode DNA samples tested from Scottish agricultural soils, 28% were determined to contain *Pasteuria* spp. Sanger sequencing of these products revealed two sequences matching to reported pathotypes, and a third dominant sequence which could not be linked to any published reports. In the soils tested, unidentified free living nematodes and *Pratylenchus* spp. encumbered with spores were recovered. Following on from this work, we have developed a high throughput metagenetic tool that takes advantage of recent advances in multiplexing technology and molecular profiling of nematode community structure, which may be used to study the interplay between *Pasteuria* spp. and nematodes communities on a small and large scale. Making use of two existing DNA sample banks, we have used this tool to: 1) study the dynamics of *Pasteuria* spp. and nematode community structure in Scottish soils; and 2) to assess any impact of abiotic factors on the ability of soils to support *Pasteuria* spp.

ESTRATEGIAS DE CONTROL DE NEMATÓDO QUISTE *GLOBODERA PALLIDA* EN MONOCULTIVO DE PAPA EN LA REGIÓN AUSTRAL DE CHILE, PROVINCIA DE MAGALLANES, TEMPORADAS 2012 A 2015. Pacheco, H.¹, I.M. Lehuédé², H. Mansilla³, R. Rodríguez³, S. Álvarez³. Servicio Agrícola y Ganadero (SAG), Chile. ¹Laboratorio de Nematología SAG Lo Aguirre; ²SAG Central, División de Protección Agrícola y Forestal; ³SAG Región de Magallanes.

En la región de Magallanes y la Antártica Chilena, ciudad de Punta Arenas ubicada en latitud 53°S, el cultivo de papa se desarrolla en condiciones de monocultivo, en siembra de primavera - verano y en ausencia de riego. El nematodo blanco quiste de la papa, *Globodera pallida* se encuentra presente en casi la totalidad de los predios destinados a producción de autoconsumo de papa y es el principal agente responsable de los bajos rendimientos obtenidos por los agricultores. Con el objetivo de enfrentar ésta problemática, el SAG estableció un ensayo para investigar estrategias para enfrentar la plaga. En un predio con un alto nivel de infestación se dispuso de 35 microparcels de 5x3 m. sobre las que se distribuyeron tratamientos con enfoque químico, biológico y mixto durante 3 temporadas de producción. La época para la aplicación de los productos fue en la siembra de tubérculos y en la aporca. Se realizó la estimación de la viabilidad de la plaga en el suelo, en condiciones de laboratorio, exponiendo los quistes a exudado de raíces de papa durante 4 semanas y que finalmente expresa en número de huevos-juveniles - viables por gramo de suelo. El muestreo de suelo para viabilidad se realizó en la época de - siembra de papa, en la aporca y a la cosecha del cultivo. Además se evaluó el rendimiento de los distintos tratamientos, separando y pesando los tubérculos en 3 calibres. Después de 3 temporadas de evaluación los resultados muestran que la viabilidad del nematodo quiste se encuentra en niveles altos en las microparcels donde hubo cultivo de papa y que recibieron algún tipo de tratamiento de control (40 huevos-J₂ g⁻¹ de suelo). La viabilidad es mayor respecto de las microparcels en que no se estableció cultivo de papa (25 huevos-J₂ g⁻¹ de suelo),- en las que la curva de declinación poblacional en 3 años no alcanza aún niveles bajos de viabilidad menores a 5 huevos-J₂ g⁻¹ de suelo. Respecto de la producción de tubérculos existió muy buena respuesta de los tratamientos, siendo la combinación de productos químicos la mejor opción, lográndose un rendimiento equivalente a 20 tonha⁻¹ de calibre comercial respecto de un cultivo sin tratamiento. Así, se ha logrado levantar evidencia que bajo las condiciones de cultivo de papa en la región de Magallanes, no es posible la erradicación de la plaga con productos no fumigantes, sin embargo, existe la posibilidad de aumentar la competitividad del rubro en la zona mediante la aplicación oportuna de alternativas de control las que se investigaran este año por medio de Proyectos de Control Alternativo para nematodos quistes de la papa (NPQ).

NEW INSIGHTS ON THE SYSTEMATICS OF THE FAMILY APORCELAIMIDAE HEYNS, 1965 (NEMATODA: DORYLAIMIDA). **Peña-Santiago, R. and S. Álvarez-Ortega.** Departamento de Biología Animal, Biología Vegetal y Ecología, Universidad de Jaén, Campus “Las Lagunillas” s/n, 23071-Jaén, Spain.

The family Aporcelaimidae, with 231 nominal and 172 valid species of 15 valid (plus two synonymous) genera, is one of the most widely spread dorylaimid group, *Aporcelaimellus obtusicaudatus* probably being the most abundant, nearly cosmopolitan, dorylaim (even nematode) on Earth. Originally, the Aporcelaimidae were characterized by the combination of several features, among others: large general size, thick cuticle, oral aperture a dorso-ventral slit, protruding stomatal structure either an axial odontostyle with large aperture or a mural tooth set on the ventral wall of stoma, guiding ring weak and plicate, didelphic females and tail short and rounded, similar in both sexes. This concept has not undergone significant changes during the past half-century. Nonetheless, the new millennium brought some relevant novelties when the first molecular data were available. Thus, Holterman *et al.* (2008), in the most comprehensive scrutiny hitherto carried out on Dorylaimida phylogeny, including LSU-rDNA sequences of two aporcelaimid genera, *Aporcelaimellus* and *Sectonema*, proved that these taxa did not share a recent common ancestor and that, as a consequence, Aporcelaimidae was not a natural (monophyletic) group. We herein provide the results derived from an exhaustive integrative study, combining both morphological and molecular information (D2-D3 segment of LSU-rDNA) of 28 species belonging to eight aporcelaimid genera. Molecular data confirm that the family Aporcelaimidae certainly is polyphyletic as it includes genera of three clades of the Dorylaimida tree with no recent common ancestor. The genera *Aporcelaimellus* and *Aporcelinus* share a peculiar differentiation of body cuticle, with a refractive inner layer, and belong to Holterman *et al.*'s clade D2, narrowly related to a complex clade D1 consisting of representatives of several families (Actinolaimidae, Dorylaimidae, Belondiridae, Mydonomidae, etc.). The genera *Aporcella* and *Tubixaba*, characterized by lacking *pars refringens vaginae*, might be related with members of clades D4 (discolaims) and D5 (*Tylencholaimus*), also without *pars refringens vaginae*, but the resolution of the tree is not satisfactory on this concern. And the genera *Aporcelaimus*, *Epacrolaimus*, *Metaporcelaimus*, and *Sectonema* form a well-supported clade (D7), whose relationships with other clades have yet to be elucidated.

A NEW CONCEPT OF *APORCELINUS GRANULIFERUS* (COBB, 1893) ANDRÁSSY, 2009 (NEMATODA: DORYLAIMIDA: APORCELAIMIDAE) AND ITS TAXONOMICAL CONSEQUENCES. **Peña-Santiago, R. and J. Abolafia.** Departamento de Biología Animal, Biología Vegetal y Ecología, Universidad de Jaén, Campus “Las Lagunillas” s/n, 23071-Jaén, Spain.

Aporcelinus granuliferus, the most repeatedly recorded species of its genus, has an intricate taxonomical history. Originally described under the genus *Dorylaimus*, it was subsequently transferred to *Eudorylaimus*, *Allodorylaimus* and *Aporcelinus*. Besides, other species, namely *Dorylaimus micrurus* Daday, 1905, *D. menzeli* Bally & Reydon, 1931, *D. yucatanensis* Chitwood, 1938 and *D. reynecki* Van der Linde, 1938, were regarded as its synonyms. In spite of its tentative wide spread and abundance, *A. granuliferus* was never the subject of a detailed morphological and morphometrical study; thus, the identity of multiple records and possible synonyms are questionable and require further research. Cobb's type material of this species is probably lost, as the oldest specimens deposited with the USDANC are those collected from several geographical areas by Thorne during the early decades of the twentieth century. The study of these specimens and an exhaustive scrutiny of the available literature about the species (and its synonyms) resulted in some relevant taxonomical novelties. (i) The concept of *A. granuliferus* is emended; as Cobb's original description, especially his measurements and illustrations, provided enough information to complete and update its diagnosis. (ii) The material studied by Thorne and Swanger (1936) is not certainly co-specific with Cobb's original one due to significant differences in their lip region broadness and odontostyle length, and probably belongs to a non-described species. (iii) The specimens (three females) deposited with USDANC are not identical to those studied by Cobb nor by Thorne and Swanger, and probably also belong to a non-described species. (iv) Both *D. yucatanensis* and *D. reynecki* are valid species, easily distinguishable from *A. granuliferus*, and should be transferred to *Aporcelinus*. (v) The available information about *D. micrurus* is not enough to provide a correct characterization of this species, and therefore it should be regarded as *inquirenda* within *Aporcelinus*. (vi) The identity of the other (many) records of *A. granuliferus* needs further evaluation by the light of the new concept of the species.

ELEMENTS FOR A CLADISTIC CLASSIFICATION OF THE DORYLAIMIDA: BODY WALL FEATURES. **Peña-Santiago, R.** Departamento de Biología Animal, Biología Vegetal y Ecología, Universidad de Jaén, Campus “Las Lagunillas” s/n, 23071-Jaén, Spain.

Leaving aside its primary subdivision into two suborders, Nygolaimina and Dorylaimina, the classification of the Dorylaimida shows many enigmas, with their superfamilies and most families probably being artificial (para- or polyphyletic) groups. Molecular data are providing fundamental information about the matter, but both the number of taxa and genes hitherto sequenced are not enough to elucidate the internal relationships of dorylaimid genera with accuracy. Cladistic principles and techniques using morphological characters have been applied to analyze the classification of particular taxa, for example the families Actinolaimidae and Longidoridae. However, no general cladistic study covering the whole order Dorylaimida is available yet. This contribution presents and discusses several features of the dorylaimid body wall that are susceptible of cladistic analysis. Several of them concern the cuticle: (i) number of layers (two → three); (ii) dorylaimoid →

leptonchid/tylencholaimid nature; (iii) absence → presence of surface longitudinal ridges; (iv) absence → presence of surface transverse and longitudinal striation; (v) absence → presence of irregularities at vulva area or at caudal region; (vi) smooth → striated inner cuticle layer; (vii) absence → presence of labial and/or post-labial sclerotization. Others refer to more specialized structures: (viii) fine → coarse body pores; (ix) lateral chord without → with gland bodies; and (x) absence → presence of dorsal cell mass at level of pharyngo-intestinal junction. The polarity (plesiomorphic → apomorphic state) of each character is established and its taxonomical weight to characterize species, genera and families is discussed.

NGS-BASED ANALYSIS OF TOMATO TRANSCRIPTOME AND IDENTIFICATION OF DIFFERENTIALLY REGULATED PATHWAYS IN ROOT-KNOT NEMATODE, MYCORRHIZA AND WATER STRESS INTERACTIONS. **Pentimone, I.¹, L.C. Rosso¹, M. Colagiero¹, P. Veronico¹, M. T. Melillo¹, F. DeLuca¹, E. Fanelli¹, R. Balestrini² and A. Ciancio¹.** ¹Istituto per la Protezione Sostenibile delle Piante (IPSP), CNR, Unit of Bari, 70126, Italy, ²IPSP, CNR, Unit of Torino, 10125, Italy.

A study was carried out on the effects of root-knot nematodes (*Meloidogyne incognita*, RKN) and the arbuscular mycorrhiza *Rhizophagus intraradices* (AM) on tomato (*Solanum lycopersicum* cv San Marzano nano), with or without water stress (WS). The RNAs were extracted and sequenced from 6-weeks-old roots (AM+ and AM-), one week after RKNs inoculation. Illumina NGS transcriptomic data were produced from all treatments. CLC™ analyses were applied to the reads obtained per treatment (range: 16.6–69.2 × 10⁶) mapping reads on the tomato genome SL2.40.26, to identify differentially expressed genes. In RKNs and control plants, 97.1 and 97.7% of reads mapped on SL2.40.26, respectively, with lower frequencies for plants combining AM, RKN and/or WS (77.7–95.0%). Mean transcripts with RPKM > 1 ranged from 18,234 (uninoculated control) to 19,964 (AM+RKNs). Gene Ontology analyses showed significantly (P < 0.01) enriched terms. Biological processes (BP) differentially up-regulated between RKN or RKN+AM plants and control included response to biotic stimulus, microtubules-based movement and lipid localization. WS additionally up-regulated BPs for localization and transport. Addition of AM to RKN-treated plants differentially up-regulated oxidation reduction and transport processes. All treatments down-regulated BP related to defense. AM additionally repressed BP for gene expression, regulation and transcription. WS repressed the regulation of macromolecule and cellular biosynthetic processes and nucleobase, nucleoside, nucleotide and nucleic acid metabolism. Molecular functions (MF) differentially up-regulated included antioxidant, catalytic and oxidoreductase (hydrolase, ferroxidase and peroxidase) activities. Plants with RKN+AM differentially up-regulated transferase activity (transferring phosphorous-containing groups), kinase, protein kinase and phosphotransferase activities. WS up-regulated molecular transducer, electron carrier, carboxylesterase, kinase and signal transducer activities. AM addition to RKN-treated plants differentially up-regulated MF like peptidase (endopeptidase), antioxidant and lipid binding. Down-regulated MFs in all treatments included catalytic, DNA binding, transferase (phosphotransferase), transcription regulator/factor and protein kinase activities. RKN+AM repressed MFs related to Ca⁺⁺ binding and phosphoric ester hydrolase. Additional WS repressed oxygen binding, ATPase coupled to transmembrane movement, and glutathione transferase. AM addition to RKN-treated plants differentially repressed lyase, transferase (glycosyl groups) and glycosyl transferase activities. A transcriptome reprogramming including the up-regulation of pathogenesis-related genes was clear for each treatment, with some up-regulated genes involved in response to environment and biotic stresses. Pathways specifically up-regulated with RKN, AM or WS included thaumatin-like proteins, induced by biotic or abiotic stress. Cell-wall metabolism genes, including expansin-encoding genes, showed differential expression, with cell wall synthesis and remodeling (cellulose synthase, pectate lyases, polygalacturonases or cell-wall degradation-related) overrepresented. The defensive phenylpropanoid pathway was strongly regulated, with cinnamyl alcohol dehydrogenase, a key enzyme in lignin biosynthesis, strongly overexpressed (47, 33 and 26-fold, respectively for RKN alone, with AM or WS). (Study partially funded by CNR, Progetto Premiale Aqua).

SUBSAMPLING AND COUNTING IN NEMATOLOGY: A CASE STUDY WITH POPULATIONS DENSITIES OF *HETERODERA GLYCINES*. **Pérez-Hernández, O.¹, A. Giri¹, F. Kidwaro¹, O. Montesinos-López².** ¹Department of Biology and Agriculture, University of Central Missouri. Warrensburg, MO 64093. ²International Maize and Wheat Improvement Center. Km. 45, Carretera México-Veracruz, El Batán, Texcoco CP 56237 Edo. de México, México

Field nematode samples typically contain more soil volume than it is feasible to process and more individuals than it is reasonable to count. Therefore, nematode abundance in a sample is based on counting individuals in representative subsamples. Information on the mean number and variability of the field sample population is thus derived from ultimate counted subsamples. This situation occurs with the estimation of the Soybean Cyst Nematode (SCN, *Heterodera glycines*) abundance in field soil samples, which are typically collected as composites (10 to 20 soil cores) and from which a subsample is processed. Further, from processed subsamples (first-order subsamples), once SCN eggs have been extracted in a certain water volume, a subsample (second-order subsample) is collected and a single reading is performed under the microscope for counting the eggs. The objectives of this study were to determine: (i) the contribution of subsampling to the estimation of SCN abundance and (ii) the error rates in detection of SCN in samples with relatively low egg concentrations. For the first objective a series of equations, originally developed for studying abundance of plankton, were used to estimate the variability added by successive subsamples, with modification to account for cyst extraction efficiency in samples with different soil

texture. The results of the first analysis will emphasize the ratio of the volume of initial samples to the volume of first- and second-order subsamples. In addition, the results will include simulations based on the assumption of random samples. For the second analysis, a hierarchical Bayesian approach and sensitivity analysis for prior determinations was conducted to estimate the error rates' posterior distributions. Under the model assumptions, the false negative rate would decrease exponentially with each additional reading. The analysis found that faulty detection of *H. glycines* occurs in single readings, and it suggests that if reliable diagnosis of a sample is desired then two or more readings will significantly minimize detection errors.

DIVERSITY, ABUNDANCE AND PREVALENCE OF NEMATODES INSIDE THE INTESTINE OF NORTH AMERICAN MILLIPEDES. **Phillips, G.¹, E.C. Bernard¹, R.M. Shelley¹, and X. Sun².** ¹Department of Entomology and Plant Pathology, University of Tennessee, 2505 E. J. Chapman Drive, 370 Plant Biotechnology Building, Knoxville, TN 37996-4560 USA, ²Statistical consultant, Research Computing Support, Office of Information and Technology, University of Tennessee, 526-A Greve Hall, 821 Volunteer Boulevard, Knoxville, TN 37996.

Nematodes inhabit a wide range of ecological niches, including the gastrointestinal tracts of both vertebrates and invertebrates. Nematodes that live in the intestines of millipedes are commensal or kleptoparasitic. These nematodes have been little studied compared to insect-parasitic nematodes. Our current research focuses on the discovery, identification and abundance of nematodes that live inside the intestines of both native North American and adventive tropical millipedes. Since 2013, 750 millipedes spanning six orders, 15 families and 55 species have been dissected and examined for nematodes and other intestinal and external parasites. Seven superfamilies of intestinal nematodes have been identified: Rhabditoidea, Diplogastroidea, Thelastomatoidea, Coronostomatoidea, Rhigonematoidea, Ransomnematoida and Dorylaimoidea. The most prevalent families are Rhigonematidae (Rhigonematoidea) and Thelastomatidae (Thelastomatoidea). Many of the dissected millipedes harbored new species, such as three new species of the rare genus *Coronostoma*. Until recently, only six species of *Coronostoma* spp. had been reported from tropical spirostreptid millipedes; we have collected this genus from one Florida and two Appalachian millipede species in the orders Spirobolida and Polydesmida. At least 20 other species of nematodes await descriptions or redescrptions from this research. Rhabditid and diplogastrid nematodes have been found in intestines only as dauer juveniles. Nematode loads have ranged from 0–1,750 per millipede, primarily located in the mid and hind intestine. Some millipede species, such as *Choctella cumminsi*, harbor only one species of nematode, while others contain as many as eight. Female nematodes generally outnumber males, and from January to July, juveniles are significantly less abundant than adults. Overall mean nematode colonization within the millipede intestine showed decreased loads between January through July and then a two-fold mean increase from July to December. Morphometric analyses using multiple regression suggest that body width and length may be limiting factors in successful intestinal colonization.

MANAGEMENT OF THE POTATO CYST NEMATODES *GLOBODERA PALLIDA* AND *G. ROSTOCHIENSIS*. **Pickup, J.** Science and Advice for Scottish Agriculture, Roddinglaw Road, Edinburgh EH12 9FJ, UK.

The two very closely related species *Globodera pallida* and *G. rostochiensis* of potato cyst nematodes co-evolved with the potato in South America. They are recognised as quarantine pests throughout the world due to the levels of loss that they can cause to potato production. In relatively recent times they have been introduced into most of the potato growing areas of the world, although reports remain scarce from some countries with extensive potato acreages, e.g. Australia, Canada, USA, India, parts of the former USSR, and they remain absent from China. Potato cyst nematodes have recently been recognised as the pest of greatest concern to potato growers within the UK, where they are widely distributed. As quarantine organisms, strict controls on the transmission of potato cyst nematodes throughout the potato propagation system are statutory requirements in most parts of the world, e.g. the measures embodied in European Directive 2007/33/EC on the control of potato cyst nematodes. Such measures have had limited success in controlling the spread of potato cyst nematodes and in many regions growers require tools to combat existing infestations. Growing resistant varieties on infested land is widely recognised as the most effective management tool. Most modern commercial potato cultivars have been bred from potato species with no resistance. However, as potato cyst nematodes have spread with potato production, breeders have turned to native South American species of *Solanum* for germplasm conferring resistance. Initially successful against *G. rostochiensis*, varieties with high resistance against *G. pallida* are becoming increasingly available. Control options, including more conventional methods such as pesticides treatments, as well as more novel methods such as trap crops and bio-fumigants are also available to growers. Understanding the effects of all available methods on population development is extremely important in achieving a sustainable control programme.

EXPERIMENTAL CHEMICAL APPLICATIONS FOR CONTROL OF CORKY RING SPOT DISEASE OF POTATO VECTORED BY *PARATRICHODORUS ALLIUS*. **Plaisance, A.¹, G.P. Yan¹, D. Peterson¹, N.C. Gudmestad¹, and K.B. Thorsness².** ¹North Dakota State University, Department of Plant Pathology, Fargo, ND 58108-6050. ²Bayer CropScience, Fargo, ND 58103.

Corky ringspot (CRS) disease on potato is caused by *Tobacco rattle virus*, which is vectored by stubby-root nematodes (*Trichodorus* and *Paratrachodorus*) and can result in up to 55% of potatoes from a harvest to be unmarketable. In 2014, dry brown necrotic arcs typical of the disease were found on tubers of potatoes in a field in Sargent County, North Dakota. In

April 2015, seven out of forty-nine soil samples from this field contained *Paratrichodorus allius* in densities ranging from 135 to 300 (mean = 175) per kg of soil. To test the effectiveness of chemical applications, six experimental treatment regimes (treatments 2 - 7) were applied to rows of a susceptible variety of potato, Yukon Gold, in this field. Each treatment regime and the non-treated control (treatment 1) had four replications (n = 28), and all rows were separated by non-treated border rows. Treatments consisted of multiple applications of insecticides (Spirotetramat and Clothianidin), a nematicide (Oxamyl), fungicides (Penthiopyrad and Fluopyram) and biological control agents (*Bacillus subtilis*, *Myrothecium verrucaria*). Disease incidence (percentage of infected tubers) and disease severity (percentage of surface area covered by brown corky lesions) were calculated from tuber subsamples (120 per treatment) at two time intervals, at harvest and again at 98 days after harvest (DAH), stored at 4.4 °C. At harvest, disease parameters (incidence, severity) for treatment 1 (10.83%, 11.38%) was not significantly different ($LSD_{P=0.05} = 9.00, 6.20$) than treatment 2 (1.66%, 3.62%), 4 (20.00%, 10.57%), 5 (14.16%, 7.52%), 6 (5.00%, 17.41%), or 7 (1.66%, 2.69%), but treatment 1 had significantly higher disease incidence than treatment 3 (0.83%, 5.33%). At 98 DAH, treatment 2 (0.83%, 0.10%) had the least disease ($LSD_{P=0.05} = 5.42, 6.35$) compared to treatment 1 (5.00%, 7.10%), 3 (7.5%, 3.57%), 4 (2.50%, 4.17%), 5 (9.16%, 8.53%), 6 (5.83%, 2.63%), and 7 (4.16%, 2.58%). Overall, Oxamyl combined with Clothianidin was most effective at controlling disease at both time intervals, but did not result in increased yield. Yield (kg) was calculated at harvest; treatment 1 (22.36) was not significantly different ($LSD_{P=0.05} = 3.97$) than treatments 2 (20.12), 3 (21.22), 4 (25.43), 5 (23.83), 6 (22.86), or 7 (23.59). Treatment 4, which contained Spirotetramat, Clothianidin, and Fluopyram, had significantly higher yield than treatments 2 and 3, which contained Oxamyl and Clothianidin. Many potato processing companies have a zero-tolerance policy for potato tubers with CRS, where an entire shipment can be rejected if a single infected tuber is identified, making disease incidence an important parameter. These experiments showed the potential for combined chemical applications to manage CRS. A second year of research at this location will be conducted in 2016 to further evaluate the effectiveness of chemical applications.

SELECTED NORTHERN-GROWN CROPS AS HOSTS OF *PRATYLENCHUS SCRIBNERI*. Plaisance, A., G.P. Yan, and A. Upadhaya. North Dakota State University, Department of Plant Pathology, Fargo, ND 58108-6050.

Many species of *Pratylenchus* are known parasites of potato, wheat, corn and soybean, causing darkened necrotic lesions on tubers and roots. In April 2015, *P. scribneri* was found to be the most prevalent plant parasitic nematode infesting a potato field in Sargent County, North Dakota. Crop species and cultivars may vary in hosting ability for this nematode and therefore were tested in this study. Four replications of four potato cultivars (All Blue, Yukon Gold, Red Norland, Russet Burbank) and two wheat cultivars (Alpowa, Louise) were planted in pots containing 1.5 kg of soil naturally infested with 1,125 *P. scribneri*, maintained at 22 °C in a greenhouse environment. Plants were harvested after ten weeks, and nematodes were extracted from soil using sugar floatation method and from roots using Whitehead tray method. Nematodes recovered from both roots and soil were added together to calculate reproduction factors (pf/pi). Reproduction factors of *P. scribneri* for Yukon Gold (6.25), All Blue (6.00), Russet Burbank (4.97), and Red Norland (2.27) were greater than Louise (0.42) and Alpowa (0.06). More nematodes were present in the soil than the roots for each cultivar; Red Norland (roots: 30.6%), Yukon Gold (16.5%), Russet Burbank (25.3%), All Blue (17.2%), Alpowa (8.1%), and Louise (7.9%). This experiment was repeated with the addition of three corn cultivars (DK-43-46, DK-43-48, DK-44-13) and two soybean cultivars (Barnes, Sheyenne). Each of the corn cultivars was artificially inoculated with 685 *P. scribneri* collected from roots of the previous experiment. Similar to the first experiment, reproduction factors of *P. scribneri* for All Blue (6.98), Red Norland (5.23), Russet Burbank (4.46), and Yukon Gold (2.90) were greater than Louise (1.04) and Alpowa (0.47). This indicates that all four potato cultivars were good (pf/pi ≥ 2.00) hosts, Louise an intermediate (pf/pi = 1.00-2.00) host, and Alpowa a poor (pf/pi ≤ 1.00) host. The reproduction factors and percent of *P. scribneri* present in the roots indicated that corn cultivars DK-44-13 (7.25, 21.8%), DK-43-46 (5.74, 25.5%), and DK-43-48 (4.72, 34.9%) were good hosts, but soybean cultivars Barnes (1.24, 23.7%) and Sheyenne (1.13, 49.1%) were intermediate hosts. Unlike the first experiment, there were more *P. scribneri* present in the roots of Alpowa (44.5%) and Louise (27.1%), but fewer present in the roots of russet Burbank (9.0%) and All Blue (8.7%), with similar number of nematodes present in the roots of Red Norland (28.6%) and Yukon Gold (17.4%). Overall, All Blue resulted in the greatest number of *P. scribneri* across both experiments. When the data for both experiments were combined, this research showed these four potato cultivars and three corn cultivars were good hosts for *P. scribneri*, but the two wheat cultivars were poor hosts, and two soybean cultivars as intermediate hosts. Greater than 50% of nematodes were present in the soil for every cultivar. A third experiment is currently being conducted to supplement these results.

HETERODERA EFFECTOR PROTEIN 4E02 IS A POWERFUL REGULATOR OF PLANT SUSCEPTIBILITY. Pogorelko, G.V., Dept of Plant Pathology and Microbiology, Iowa State University, Ames, IA 50011.

Cyst nematodes invade host plant roots and orchestrate dramatic cellular changes to form elaborate feeding sites. Esophageal gland-produced and stylet-secreted effector proteins play important roles during this complex plant-nematode interaction and drive the parasitic success of these pathogens. Effector 4E02 is produced in the subventral esophageal gland cells of both *Heterodera glycines* and *H. schachtii* cyst nematodes. We have utilized the Arabidopsis-*H. schachtii* pathosystem to conduct detailed functional characterization of this effector. Constitutive expression in Arabidopsis of the

H. schachtii 4E02 coding sequence without the nematode secretion signal peptide resulted in altered expression of defense genes. Interestingly, while the pathogenesis-related genes *PAD4* and *WRKY40* were upregulated, *PDF1.2* was significantly down-regulated. In addition, we determined that cell wall composition of these transgenic Arabidopsis lines was altered in that mannose and glucuronic acid content increased specifically in roots while rhamnose and galacturonic acid contents decreased in shoots. These changes were accompanied by increased susceptibility to *H. schachtii* and the necrotrophic fungal pathogen *Botrytis cinerea* suggesting an important virulence function of 4E02. Using *in vitro* and *in vivo* methods, we determined that the *H. schachtii* 4E02 protein specifically interacts with Arabidopsis vacuolar papain-like cysteine protease RD21A, which has been reported to play important roles in different pathosystems. Promoter and gene activity studies revealed that *RD21A* expression is specifically up-regulated in the syncytium at the early stages of infection. Activity-based protein profiling indicated that RD21A protein activity was not altered by 4E02. However, in the presence of 4E02, RD21A localized to the plant nucleus and the cytoplasm instead of the vacuole, suggesting altered targeting of this known defense regulator as the mode-of-action of the 4E02 effector. A yeast 2-hybrid screen using RD21A as bait identified multiple interacting proteins that are known to function in PAMP-triggered and effector-triggered immunity. Our data establish the 4E02 effector as a major modulator of plant susceptibility that acts by altering localization of a defense-regulating plant protein.

META-ANALYSIS OF THE INFLUENCE OF AGRICULTURAL INTENSIFICATION AND URBANIZATION ON NEMATODE DIVERSITY. Pothula, S.K.¹, P.S. Grewal⁴, R.M. Auge², A.M. Saxton³, and E.C. Bernard¹. ¹Department of Entomology and Plant Pathology, ²Department of Plant Sciences, ³Animal Science, University of Tennessee, Knoxville TN 37996 USA, ⁴School of Earth, Environmental, and Marine Sciences, University of Texas Rio Grande Valley, 1201 West University Drive, Edinburg, Texas 78539-2999 USA.

Nematodes are at a central place in the soil food web. The structure of nematode communities provides useful information on the condition of the soil food web. Our aim was to determine the effect of human interference on the diversity and abundance of nematodes found in different ecosystems. Meta-analysis was conducted using comprehensive meta-analysis software to compare the diversity and abundance of nematode communities according to trophic and colonizer-persister (CP) groups among urban, agricultural, and forest ecosystems. A total of 539 relevant articles were found by using a sequence of different search terms, out of which 30 articles were selected for this preliminary analysis. Our results indicate that nematode genus diversity in omnivores, predators, plant feeders, fungivores, and bacterivores per 100g of soil is higher in forest ecosystems compared to agricultural and urban ecosystems. Similarly, nematode genus diversity in CP 2, CP 3, CP 4, and CP 5 is higher in forest ecosystems compared to agricultural and urban ecosystems. In contrast, total nematode abundance was significantly higher in agricultural ecosystems than in forest and urban ecosystems because of higher abundance of lower trophic and CP groups indicating disturbance of the soil food web. Agricultural intensification and urbanization apparently negatively impact nematode community diversity that is critical for the maintenance of soil ecosystem services and resilience.

ADDING PLANT PARASITIC NEMATODES TO THE BARCODE OF LIFE DATABASE. Powers, T., R.S. Higgins, T.S. Harris, P.G. Mullin, and K.S. Powers. Department of Plant Pathology, University of Nebraska-Lincoln, Lincoln NE 68583.

The Barcode of Life Database (BOLD) is a DNA-based repository and workspace designed to explore species identity and diversity. There are public and private records maintained on the database, and mechanisms are built-in to facilitate data-sharing for collaborative work. Currently there are 559 species with barcodes representing Nematoda, less than 2.0% of the total described species in the phylum. This places nematodes among the most poorly characterized organisms in the database. This paucity of nematode barcodes may be linked to historical difficulties in PCR amplification of COI, however sufficient nematode sequence data are now available for primer design across a broad range of taxa. We have recently deposited over 1,300 barcode sequences of nematodes in Criconematina. These barcodes represent 94 well-supported clades or clusters, 46 named species, and 25 singletons which do not associate with any cluster. The bulk of the dataset are from North America, but specimens from Africa, Asia, Australia, Central America, and Europe are included. A strong geographic signal is apparent in the distribution of barcodes supporting the use of this marker for biogeographic and phylogeographic studies. The DNA barcodes are not meant to supplant taxonomy; rather, a well-curated, taxonomically validated set of barcoded specimens should enhance applications such as identification and biodiversity assessment.

COMPARATIVE TAXONOMIC RESOLUTION OF GENETIC MARKERS FOR DNA BARCODING AND CLASSIFICATION: A CRICONEMATID MODEL. Powers, T., R.S. Higgins, T.S. Harris, P.G. Mullin, and K.S. Powers. Department of Plant Pathology, University of Nebraska-Lincoln, Lincoln NE 68583.

Nematodes in the suborder Criconematina are an excellent model system for evaluating morphological and molecular characters in species delimitation and classification. Strong support exists for the overall monophyletic status of the group. The existence of species associated with native plant communities as well as agroecosystems permits the investigation of the role of geographic isolation in nematode differentiation. Criconematid nematodes have limited dispersal capabilities and

many of the morphological characters used to define genera and species are derived from the cuticle and are easy to observe. Our molecular analyses using 18S, ITS1, and the mitochondrial barcode gene COI suggest that genera and species defined on the basis of morphological characters are not well-supported. Molecular analyses raise questions such as: Can a species be a *Lobocriconema* without lobes? Is there a short-stylet form of *Mesocriconema xenoplax*? To what extent does the arrangement of scales on the cuticle reflect groupings based on DNA? At this point in our analyses, it appears that there is extensive convergent evolution and homoplasy in traditional classifications of Criconematina. COI as a genetic marker reveals nearly two orders of magnitude more variation than 18S. As a taxonomic marker COI is informative at the population and species level, but less informative at deeper nodes in the phylogenetic tree. Support for the deeper nodes in the tree is improved with the addition of more slowly evolving genes such as the 18S or 28S ribosomal genes.

KNOWN AND NOVEL FUNGI COLONIZING SOYBEAN CYST NEMATODE CYSTS – SEQUENCING THE COMMUNITIES. **Rajendran, D.¹, Y. Zhu², W. Hu², S. Chen^{1,2} and K.E. Bushley³.** ¹University of Minnesota, Department of Plant Pathology, 1991 Upper Buford Circle, St. Paul, MN 55108, USA. ²University of Minnesota Southern Research and Outreach Center, 35838 120th St., Waseca, MN 56093, USA, ³University of Minnesota, Department of Plant Biology, 1479 Gortner Avenue, St. Paul, MN 55108, USA.

Understanding the environment in which biocontrol needs to be elicited is critical to ensure successful establishment of the biocontrol agent in the environment. In *Glycine max* (soybean)-*Heterodera glycines* (Soybean Cyst Nematode – SCN) pathosystem, several fungal species have been reported worldwide to be effective biocontrol agents, mostly parasites of SCN second-stage juveniles (J2) or eggs. In this study, cyst-colonizing fungi collected from plots subjected to different crop sequences were identified over two crop seasons in 2014 and 2015 using their ITS1 sequences. Relative abundances of community members were also studied. The major players in the field were *Fusarium* spp., *Dactylonectria/Ilyonectria* spp., and *Cylindrocarpon* spp. Their relative percentage colonization of the cysts when compared to the other species remained high in the two years. *Lachnum* spp, *Exophiala* spp. and *Mariannea* spp. were less abundant, but consistently found in the field. Several unknown fungi (<97% query cover and/or <97% sequence identity to database listings) were also observed. This is an on-going research project, and the diversity of fungi will be analyzed across different crop sequences and seasons.

POTENTIAL ROLE OF NODULATION GENES IN ESTABLISHMENT OF FEEDING SITES INDUCED BY RENIFORM NEMATODE. **Redding, N.¹, P. Agudelo¹, and C.E. Wells².** ¹Department of Plant and Environmental Sciences and ²Department of Biological Sciences, Clemson University, Clemson, South Carolina, USA 29634.

Reniform nematode, *Rotylenchulus reniformis*, is a semi-endoparasite that infects more than 300 plant species in tropical, subtropical, and warm temperate regions worldwide. During infection, female nematodes penetrate roots to the endodermis and establish complex, multinucleate feeding sites called syncytia. We performed an RNAseq study using a soybean split-root system to identify genes involved in the process of syncytium formation. Infected and uninfected root samples were taken from three individual plants at three, six, nine, and twelve days after inoculation and used for RNA extraction and cDNA library preparation. Sequenced

Illumina reads were mapped to the soybean reference genome to estimate transcript abundance and investigate differential gene expression between treatments. All transcripts were annotated with sequence descriptions, GO terms, and enzyme codes using Blast2GO software. Gene Set Enrichment Analysis was used to identify GO terms and enzyme codes enriched in the inoculated root samples. Multiple differentially expressed genes were identified with significantly enriched GO terms involved in nitrogen fixation and nodulation. Of particular interest was the apparent involvement of CYCLOPS, NSP1, NSP2, and NIN transcription factors that are known to control nodule initiation and morphogenesis. Transcripts for Sec14 family membrane trafficking proteins and numerous nodulin-like sugar transporters, including SWEET family proteins and sugar/proton symporters, also showed expression change in infected roots. These results highlight potential commonalities between rhizobia-soybean mutualism and reniform nematode-soybean parasitism. Candidate genes identified here will be analyzed in additional functional and localization studies to confirm their role in reniform syncytium development.

VARIATION AMONG MELOIDOGYNE SPP. ISOLATES ON A PANEL OF RESISTANT CARROT GENOTYPES. **Roberts, P.A.¹, W.C. Matthews¹, P.S. Simon² and T.T. Duong¹.** ¹Department of Nematology, University of California, Riverside, CA 92521, ²USDA ARS and Department of Horticulture, University of Wisconsin, Madison, WI 53706.

A collection of 49 isolates of *Meloidogyne arenaria*, *M. hapla*, *M. incognita* and *M. javanica* were compared for their infection potential on a panel of 11 diverse sources of resistant carrots (*Daucus carota*). The resistant genotypes were sources from ‘Brasilia,’ ‘Homs,’ ‘Ping Ding’ and ‘Western Red’ or combinations of these sources. They are known to contain genes for resistance to *M. incognita* and *M. javanica*. The goal was to determine the breadth of utility of resistance traits available in carrot germplasm and whether nematode virulence to the resistance is present. A susceptible carrot, Imperator 58, was included as a control. All nematode isolates were cultured on greenhouse-grown susceptible tomato host plants. Carrots were direct-seeded into sand-filled pots and thinned to one plant per pot after emergence. One month after seeding at the 3- to 5-leaf stage, each plant root-zone was inoculated with 50,000 freshly extracted eggs. Carrot root systems were assayed for

root-galling (scale 0 – 8) and egg production 70 days after inoculation. Each isolate x carrot genotype combination was replicated four times and the test was conducted twice. The most resistant genotypes across isolates were derived from Brasilia 1252 and HxB, a cross between Homs and Brasilia. Ping Ding and Western Red also exhibited effective resistance across isolates. Of 29 *M. incognita* isolates, three were slightly more aggressive on Homs and Ping Ding, whereas the Brasilia sources were unaffected by those more aggressive isolates. The *M. incognita* isolates included ones known to be virulent on the tomato *Mi-1* gene or the cowpea *Rk* gene, but there was no correlation between virulence on the *Mi-1* and *Rk* genes and ability to parasitize resistant carrot genotypes. Two isolates of *M. arenaria* and seven isolates of *M. javanica* were avirulent on the carrot resistance sources, whereas variation in ability to parasitize resistant carrots was found among 11 *M. hapla* isolates.

IMPACT OF CONCURRENT INFECTION BY *PRATYLENCHUS PENETRANS* AND *FUSARIUM VERTICILLIOIDES* ON CORN SEEDLINGS. Rush, T.A. and A.E. MacGuidwin. Plant Pathology Dept., University of Wisconsin-Madison, Madison, WI 53706.

Pratylenchus penetrans (Pp) and *Fusarium verticillioides* (Fv) are soilborne pathogens of corn. Infection by either pathogen damages roots and can inhibit plant growth and development with the potential to reduce yield. The severity and time course of symptoms caused by a fungal potato pathogen are increased when potato is infected by population densities of Pp too low to cause disease and it has been reported this phenomenon extends to the Fusarium-corn pathosystem. Our objective was to determine the fate of corn seedlings infected by both pathogens soon after planting using a scenario of low disease pressure for Pp. Four treatments, 1) non infested control, 2) Pp only, 3) Fv only, and 4) Pp+Fv were replicated six times in a complete randomized block design for two hybrids, Pioneer 9910X and Pioneer 1105YHR. Seeds were surface sterilized and pre-germinated. Treatments receiving Fv were soaked in a suspension of conidia or sterile water for 24 hrs before planting in cones filled with 700cm³ of pasteurized loamy sand soil. The Fv isolated, transformed to express GFP, was obtained from Iowa State University. Inoculum of 1000 Pp in water or water only was added to the hole before placing the seed. Nematodes were recovered from monoxenic root explant cultures by incubating roots on Baermann funnels. Plants were grown in a growth chamber under a, 14- hr photoperiod at 28 C and watered daily starting at 7 days after planting. Plant height, measured from the soil line to the tip of the tallest leaf, was recorded every day and used to calculate an area under the growth curve (AUGC). Thirty days after planting, plants were harvested and data collected for fresh and dry weight of plant organs. Nematodes were recovered from soil and roots and infection by the fungus was verified using fluorescence microscopy of plant tissues and morphology of conidia isolated from roots, stems and first leaf. The experiment was repeated twice and the data analyzed using SAS PROC MIXED. The Pioneer 9910X hybrid exposed to Fv only had lower ($P < 0.05$) dry root weights and lower values for AUGC as compared to the other three treatments. There were no differences between the Pp+Fv and control plants, indicating that concurrent infection by Pp mitigated the effect of the fungus. Absolute population densities of Pp per cone (roots and soil) and Pp per gram of dry root were not different for the Pp and Pp+Fv treatments. The Fv fungus was only recovered from plants receiving the conidial inoculum. No effects of any treatment were detected for the Pioneer 1105YHR hybrid. Studies are in progress to determine if Pp and Fv interact during the infection and colonization processes.

TRANSCRIPTOMIC PARTICULARITIES AMONG SPECIES OF *GLOBODERA*. Sabeh, M.^{1,3}, É. Grenier², M. St-Arnaud³, and B. Mimee¹. ¹Saint-Jean-sur-Richelieu Research and Development Center, Agriculture and Agri-Food Canada, Saint-Jean-sur-Richelieu (Qc), Canada J3B3E6; ²Institut National de Recherche Agronomique, Biologie des Organismes et des Populations appliquée à la Protection des Plantes, Domaine de la Motte, BP 35327, 35653 Le Rheu cedex France; ³Institut de Recherche en Biologie Végétale, Université de Montréal and Jardin Botanique de Montréal, Montréal (Qc), Canada H1X2B2.

Globodera spp. are major plant parasitic nematodes affecting Solanaceous plants including potato, tomato, eggplant and tobacco. Each species secretes specialized proteins called *effectors* to outwit plant defenses, allowing them to have a unique host range. RNA sequencing of the J2 parasitic stage was used to identify sequence variations and expression differences in effector genes that could explain species specificity for their host. *G. rostochiensis*, *G. pallida*, *G. mexicana* and *G. tabacum* were chosen for their economic importance, distinctive host range and high genetic similarity. Two distinct populations of each species were used to avoid finding intra-species variations. *G. rostochiensis* was directly compared to *G. tabacum* because of the difference in their primary host (*S. tuberosum* and *N. tabacum*) and *G. pallida* to *G. mexicana* because of the broader host range of *G. pallida*. RNA sequencing was performed on an Illumina HiSeq2500 producing an average of 29M sequenced 125bp paired-reads per sample. Data analysis was achieved using CLC Genomics Workbench. Clustering of gene expression patterns highlighted several differentially expressed effector genes. SPRYSEC and RBP-1 genes were overexpressed in *G. rostochiensis* compared to *G. tabacum*. On the other hand, CLE-1 and SKP-1 were overexpressed in *G. tabacum*. For the other pair, pectase lyase genes and ENG-3 were overexpressed in *G. pallida* while Gpx and CM-1 were overexpressed in *G. mexicana*. For sequences variations, only large variation (≥ 3) in effector genes sequences were searched for, as they are more prone to affect the molecular interactions of the protein. Fairly large insertions (8 and 5 bp) were found in *G. rostochiensis* SPRYSEC-4 and -5 as compared with its orthologue in *G. tabacum*. Important variations were also found in *G. pallida*'s IC5, SPRYSEC-1 and -19 genes as well as in pectase lyase when compared with orthologues in *G. mexicana*.

FIRST RECORD OF *DIPLOSCAPTER CORONATA* (NEMATODA: RHABDITIDAE), A FACULTATIVE PARASITE OF MAN, ASSOCIATED WITH TOMATO CROPS IN ARGENTINA. **Salas, A., J.M. Rusconi, D. Eliceche, and M.F. Achinelly.** Centro de Estudios Parasitológicos y de Vectores, Facultad de Ciencias Naturales y Museo, (UNLP), CONICET. Argentina.

Diploscapter coronata (Cobb, 1893) Cobb, 1913, is a free-living soil bacterial-feeding nematode, found in compost, sewage or agricultural soil and as facultative parasite of insects and vertebrates, even humans. The clinical symptoms include epigastric tenderness, diarrhea, crampy abdominal pain, weakness and nausea. They have been considered as a potential carrier of bacteria pathogenic to the surface of preharvest fruits and vegetables in contact with soil. In this note we reported the presence of *D. coronata* in the framework of different agro-ecological labors in Argentina. Soil samples associated with tomato crops (*Lycopersicon esculentum*) were taken and processed by the centrifugation method to isolated nematodes. Specimens were identified by morphological and morphometric characteristics of females. *Diploscapter coronata* was characterized by annulated cuticle, two pairs of lips in the mouth region, lips transformed into a pair of medial, outwardly acting, distally bifurcate fosses and a pair of lateral lamellae, rhabditoid type of oral cavity, absence of glottoid apparatus. The detection of this nematode in greenhouses where dogs, cats and poultry live together without any health control, highlight the importance of applying proper hygiene measures during agricultural practices to prevention of infections in vertebrates and humans. This report constitutes the first record of this nematode species and genus for Argentina.

NEMATODE DIVERSITY AND SOIL FOOD WEB MODELLING: TOOLS, SCENARIOS, AND PERSPECTIVES. **Sánchez-Moreno, S.** Plant Protection Products Units (DTEVPF). National Institute for Agricultural and Food Research and Technology, Madrid, Spain.

In 25 years, contemporary nematode ecology has been born, developed, and grown to become a solid scientific discipline, providing unique tools for analysing soil food web condition in a number of habitats and scenarios. Unlike other soil organisms, nematode communities provide high-quality ecological data, which allow inferences about soil food web structuring forces, stoichiometry, and multitrophic interactions in agricultural and non-agricultural systems. Nevertheless, nematode ecology has not been widely included in soil ecology studies. Free availability of on-line databases on nematode ecophysiological parameters and on-line indicator calculators are unique tools that allow analysis of complex nematode data with minimal effort, and should be seen by nematologists as an opportunity to increase the impact of nematode studies in soil sciences. Together with recent statistical analyses such as Structural Equation Modelling, such tools are opening a new era in which nematode ecology will play a significant role in food web studies.

IDENTIFICACIÓN MOLECULAR DE ESPECIES DE *PRATYLENCHUS* ASOCIADAS A CULTIVOS AGRÍCOLAS DE COSTA RICA. **Sandoval-Ruiz, R.¹, L. Flores-Chaves¹, D.A. Humphreys-Pereira¹, and L. Gómez-Alpizar².** ¹Laboratorio de Nematología-CIPROC, Universidad de Costa Rica, 2060 San Pedro, Costa Rica, ²Laboratorio de Biotecnología de Plantas-CIA, Universidad de Costa Rica, 2060 San Pedro, Costa Rica.

Pratylenchus es un género estenomórfico, polígrafo, con especies que abarcan una amplia diversidad de ambientes y es causante de importantes pérdidas económicas en el mundo. La información referente a la identificación de especies de *Pratylenchus* presentes en cultivos agrícolas de Costa Rica es escasa. El objetivo de este trabajo fue determinar por medio de técnicas moleculares, PCR y secuenciación de la región D3 del gen 28S del ADN ribosomal (ADNr) las especies del género *Pratylenchus* asociadas a 12 cultivos agrícolas de Costa Rica. Las muestras se recolectaron en al menos una finca por cultivo, mínimo tres muestras compuestas, cada una conformada por 10 submuestras. Los cultivos analizados fueron: en la provincia de Alajuela: arroz de Quebradón de Upala (PA), pimienta de Muelle de San Carlos (PM) y caña de azúcar de Grecia (PÑ1); en la provincia de San José: áster de Puriscal (PS) y café de San Marcos de Tarrazú (PC2); en la provincia de Heredia: banano de Sarapiquí (PB) y lirio (PL); en la provincia de Cartago: gypsophila (PG), café de Navarro de Orosi (PC1), cebolla (PE1) y papa (PP) de San Juan de Chicué, cebolla (PE2) y un cultivo mixto de papa- cebolla (PP-E) de Pacayas; fresa de Llano Grande, (PF); helecho hoja de cuero de BIRRISITO (PH); en la provincia de Guanacaste: caña de azúcar de Cañas (PÑ2). La extracción de ADN de *Pratylenchus* se llevó a cabo en WLB (Worm Lysis Buffer) + proteinasa K. Para la amplificación de ADN se utilizaron los imprimadores D3A/D3B. Las secuencias obtenidas se compararon por medio de Blast Search con las de *Pratylenchus* spp. previamente depositadas en el GenBank. Con el fin de corroborar la identidad de las especies se realizó un análisis filogenético, con el criterio de Máxima Verosimilitud (ML), con las secuencias obtenidas y secuencias de especies de referencia depositadas en el GenBank. El análisis filogenético de *Pratylenchus* spp. agrupó las especies de *Pratylenchus* en un clado principal. Las secuencias de las muestras PA y PÑ2 se asociaron con accesiones de *P. zae* (96%), las de PM con *P. brachyurus* (99%), PS con *P. pseudocoffeae* (99%), PC1 y PC2 con *P. gutierrezii* (99%), PE2, PL, PG y PF con *P. penetrans* (99%). Las secuencias de PE1, PP y PP-E forman un grupo separado junto con accesiones de *P. crenatus* (99%), sin embargo, las accesiones de *P. crenatus* forman un subgrupo con un valor de bootstrap de 87%. Las secuencias de PH y secuencias de PP se agruparon con *P. bolivianus* (91%). Las secuencias de PB se agruparon con accesiones de *P. speijeri*; pero se separaron de las utilizadas para describir la especie (89%). Las secuencias de PÑ1 se asociaron en igual medida a accesiones de *P. floridensis*, *P. hippaestri*, *P. paraflorendensis* y *Pratylenchus* sp. (< 80%).

ACTIVITY OF THE NEW BIONEMATICIDE MAJESTENE™ FOR CONTROLLING PLANT PARASITIC NEMATODES: CASE STUDIES. **Santos, B.M.¹, T.B. Johnson, M.J. O'Neal, and P.G. Marrone.** ¹Marrone Bio Innovations, 1540 Drew Ave., Davis, CA 95618, USA.

Controlling parasitic nematodes has become a major issue in high-value crops, mainly due to: a) inconsistent activity from various conventional soil fumigants, b) increased scrutiny and paperwork for application of soil fumigants as related to worker safety, c) lack of availability of current alternatives, and d) high nematode pressure in fields that were formerly controlled with methyl bromide. At the same time, there is considerable public pressure to find new environmentally-friendly products that could replace conventional chemistries. The bionematicide Majestene is a liquid formulation resulting from heat-killed cells of the new bacterium *Burkholderia rinojensis* strain A396 that prevents nematode molting and egg mass formation. A summary of results of independently-conducted studies in bananas and tomato is presented. In bananas, four studies were conducted between 2014 and 2015 in mature fields heavily infested with *Radopholus* spp. and *Pratylenchus* spp. All nematicides were applied in drench once during the season using a total volume of 1 L/plant. In 2014, the treatments were: 1) non-treated control, 2) oxamyl (=Vydate 24LS) at 7 mL/plant, 3) Majestene at 9.5 gal/acre, and 4) Majestene at 19 gal/acre. In 2015, the treatments were 1) non-treated control, 2) *Pochonia chlamydosporia* var. *catenulata* (=Klamic) at 313 g/ha, 3) Majestene at 9.5 gal/acre, and 4) Majestene at 19 gal/acre. Results showed no significant differences between oxamyl and both Majestene rates on the root counts of both nematode genera at 45 days after application, all of which outperformed the non-treated control. Similarly, all treated plots had superior control of *Radopholus* and *Pratylenchus* in comparison to the non-treated plots in 2015. In tomato, several studies were conducted in 2015 to compare control of *Meloidogyne* spp. with one or two drip-injections of Majestene at 9.5 and 19 L/ha, individually against metam potassium (=K-Pam or Sectagon) at 568 L/ha, *Paecilomyces lilacinus* strain 251 (=MeloCon) at 2.25 kg/ha, oxamyl (=Vydate L) at 4.7 L/ha. Data showed equal or superior control of juveniles and adults of southern root-knot nematode in all three studies. These results indicate that Majestene is a new valuable tool to control troublesome plant parasitic nematodes, while reducing the risk for personnel exposure and pest resistance. More testing is underway to: a) specify the mode of action of the product and b) expand data pool in other high-value crops in the USA and abroad.

GENETIC SCREENING OF *PHOTORHABDUS LUMINESCENS* USING *C. ELEGANS* REVEALED VITAMIN B6 BIOSYNTHETIC PATHWAYS AS AN ESSENTIAL PATHOGENIC FACTOR. **Sato, K.^{1,2}, T. Yoshiga^{1,2}, and K. Hasegawa³.** ¹Laboratory of Nematology, Department of Applied Biological Sciences, Saga University, Saga, 840-8502 Japan, ²The United Graduate School of Agricultural Sciences, Kagoshima University, Kagoshima, 890-8580 Japan, ³Department of Environmental Biology, College of Bioscience & Biotechnology, Chubu University, 1200 Matsumoto, Kasugai, 487-8501 Japan.

Photorhabdus luminescens is a Gram-negative entomopathogenic bacterium, which symbiotically associates with the entomopathogenic nematode (EPN) *Heterorhabditis bacteriophora*. *P. luminescens* is highly virulent to many insects and non-symbiotic nematodes including *Caenorhabditis elegans*; *C. elegans* L1 larva is not able to develop completely on the lawn of *P. luminescens*. To explore new pathogenic factors of *P. luminescens*, we constructed a transposon-inserted mutant library and screened for virulence deficient mutants against *C. elegans*. From the mutant screening, we identified the *pdxB* gene encoding erythronate-4-phosphate dehydrogenase, which is known to contribute toward de novo vitamin B6 biosynthesis. *pdxB* mutant showed growth deficiency in nutrient-poor medium, and such phenotype was restored by supplementation of pyridoxal 5'-phosphate (PLP), an active form of vitamin B6. Additionally, the supplementation of other three B6 vitamers (pyridoxal, pyridoxine and pyridoxamine) also restored the growth of *pdxB* mutant in nutrient-poor medium. This indicates that *P. luminescens* has a salvage pathway that can compensate for the de novo vitamin B6 biosynthesis pathway. PLP supplementation also restored the virulence of *pdxB* mutant to *C. elegans*. Furthermore, the injection of bacterial cells into superworm *Zophobas morio* revealed that the insecticidal activity of *pdxB* mutant was lower than the wild type strain. From these results, we concluded that vitamin B6 production is an essential factor for full pathogenicity toward insects and the *C. elegans*. This is our first step to understand how EPNs established a symbiotic relationship with such highly pathogenic bacterium during their evolution as well as to understand the pathogenic mechanism of this bacterium.

NEMATODE PHOSPHODIESTERASES ARE PROMISING TARGETS FOR NOVEL NEMATICIDES. **Schuster, K.¹, K.B. Cahill¹, A.S. Parker¹, W. Danquah², V.M. Williamson², and R.H. Cote¹.** ¹Univ. New Hampshire, Dept. Molecular, Cellular, & Biological Sciences, Durham NH 03824 and ²Univ. California-Davis, Dept. Plant Pathology, Davis CA 95616.

Plant-parasitic nematodes are a leading cause of reduced agricultural productivity resulting in nearly \$100 billion in damage annually in the United States. Current methods of managing these pests are either ineffective or unsafe. As a result, new methods must be developed to reduce the damage caused by these widespread pests. The central hypothesis of this research is to demonstrate the potential of using phosphodiesterase (PDE) inhibitors as a novel nematicide that can be optimized to selectively inhibit plant-parasitic nematode PDEs. The use of PDE inhibitors in human therapy have led to great advances in our understanding of how novel compounds can be developed that preferentially target one PDE family over another. This has been accomplished despite the fact that the PDE superfamily all share a highly conserved catalytic domain. This knowledge can be used to aid in the design of novel compounds that can be used as a highly selective nematicide. Our

research has found that all nematode clades contain the same set of six PDE families which are orthologous to vertebrate PDE families 1, 2, 3, 4, 8, and 10. We then characterized the pharmacological properties of nematode PDE4 in relation to vertebrate PDE4 both *in silico* and *in vitro*. Our *in silico* analysis employed evolutionary trace, homology modeling, and compound docking to identify similarities and differences in amino acid residues necessary for inhibitor binding. We then expressed nematode and human PDE4 to demonstrate that they exhibit altered pharmacological properties. We have also investigated the effects of PDE inhibitors on the behavior of *C. elegans* and determined that both pan-specific and family-specific PDE inhibitors can affect nematode locomotion. Furthermore, treatment with certain PDE inhibitors impairs the ability of the plant-parasitic root-knot nematode (*Meloidogyne hapla*) to infect plant roots. These results suggest that the use of phosphodiesterase inhibitors as nematicides could be successful in reducing crop loss as well as eliminating adverse environmental effects commonly associated with current nematicides.

COMPARATIVE HOST RESPONSES IN SUSCEPTIBLE AND RESISTANT CUCURBITACEOUS PLANTS INFECTED BY *MELOIDOGYNE INCOGNITA* AND *FUSARIUM* SPP. **Seo, Y.¹ and Y.H. Kim¹.** ¹Department of Agricultural Biotechnology and Research Institute of Agriculture and Life Sciences, Seoul National University, Seoul 151-921, Korea.

Root-knot nematode (RKN) (*Meloidogyne incognita*) and fusarium wilt (or rot) fungi (*Fusarium* spp.) cause severe damage to cucurbitaceous vegetable crops of oriental melon, cucumber and watermelon, that are mostly grown in greenhouses in Korea. Shintosa (*Cucurbit maxima* x *C. moschata*) is used commonly as a rootstock for grafting these crops because of its resistance to fusarium wilt; however, this disease is still prevalent especially in oriental melon. This study aimed at screening the four cucurbit plants for their resistance and/or susceptibility to RKN and *Fusarium* spp. that are isolated from the oriental melon fields. All cucurbit plants tested were susceptible to the *M.incognita*, showing the gall index (GI) of 3.0~3.8 and egg-mass index (EI) of 4.3~4.8 with no significant differences among the crops tested at $P \leq 0.05$ by least significant difference test (LSD). Disease severities in the four cucurbit plants caused by 29 *Fusarium* isolates varied depending on the plants and the *Fusarium* isolates, among which the most virulent isolate identified as *F. proliferatum* F6 (FP6) was highly virulent to all the crops except the shintosa that was shown to be susceptible to this fungus. Also the second-most virulent *F. oxysporum* F5 (FO5) showed virulence to the three cucurbitaceous crops but not to the shintosa, regardless of the higher and lower inoculum concentrations. On the other hand, the oriental melon and watermelon wilt fungi, *F. oxysporum* f.sp. *melonis* (FOM) and *F. oxysporum* f.sp. *nivenum* (FON) were more virulent to the oriental melon and watermelon than the cucumber, but not to the shintosa with no or minimal disease index of the fusarium wilt (or rot) (DI). In the four crops above infected with both RKN and the fungal pathogen, GI was mostly reduced more greatly, EI less greatly, but not DI with even increased disease severities in watermelon with FO5 and oriental melon with FP6. The fungal pathogen invasion (PI) and the degrees of giant cell formation and destruction were examined by the light microscopy, which appeared to be largely related to DI and EI (especially in the shintosa), respectively. The PI increased in the root-stock shintosa by the coinoculation suggests the increase of DI by the multiple infection in the scion crops susceptible to the fusarium wilt (or rot) by the spread of virulence factors through the vascular tissue system.

IPR 106: NEW ARABICA COFFEE CULTIVAR WITH SIMULTANEOUS RESISTANCE TO *Meloidogyne paranaensis* AND *M. incognita*. **Sera, G.H.¹, T. Sera¹, A.C.Z. Machado², S.A. Silva², D.S. Ito², and L.H. Shigueoka².** ¹ Plant Breeding Dept., Instituto Agronômico do Paraná, Londrina, PR, Brazil, ² Plant Protection Dept., Instituto Agronômico do Paraná, Londrina, PR, Brazil.

The main coffee-parasitic nematodes in Brazil are *Meloidogyne paranaensis* and *M. incognita*, which exhibit aggressive behavior that prevents the establishment of plantations, and *M. exigua*, which is important because of its widespread geographical distribution. The use of cultivars resistant to these nematodes is the best choice in infested areas, because it represents a control method more efficient, economically viable and environmentally correct. However, there are few coffee cultivars available with resistance to nematodes. Current recommendations for infested areas include hypocotyledonary grafting, using the rootstock cultivar Apoatã IAC 2258 (*Coffea canephora*) that is resistant to *M. exigua*, *M. incognita* and *M. paranaensis*. The cultivar IPR 100 is the only Arabica coffee recommended for infested areas with these nematodes in Brazil. The aim of this study was to assess IPR 106 Arabica coffee cultivar for resistance to *M. paranaensis* and *M. incognita*. Experiments to assess the resistance to nematodes *M. paranaensis* and *M. incognita* were conducted in a greenhouse at the Instituto Agronômico do Paraná (IAPAR) in Londrina, PR, Brazil, between February and June 2014. Seedlings with four pairs of leaves planted in plastic cups with 700 ml, were inoculated with 2,000 eggs per plant. IPR 106 was compared with the susceptible check *C. arabica* cv. Catuaí Vermelho IAC 81. The experimental design in both experiments was completely randomized, with 15 replications for *M. paranaensis* and 30 replications for *M. incognita*, containing one plant per plot. The assessments were made 90 days after inoculation. The reproduction factor (RF) and number of eggs and second stage juveniles per gram of roots were used to determine the resistance reaction. To classify the resistance level of IPR 106, the reduction in reproduction factor (RRF) based on the formula: $RRF = [(RF \text{ of the susceptible control} - RF \text{ of the IPR 106}) / RF \text{ of the susceptible control}] \times 100$ was used. IPR 106 presented lower RF and nematodes/g than the susceptible control for the two nematodes. The RF of IPR 106 for *M. paranaensis* and *M. incognita* were 0.082 and 0.888, respectively, while for

susceptible control were 32.81 and 27.43. In IPR 106 were found 15 and 184 nematodes/g of *M. paranaensis* and *M. incognita*, respectively, whereas the susceptible control were 8,780 and 5,618. 'IPR 106' was classified as highly resistant to *M. paranaensis* and *M. incognita*, because it showed 99.75 and 96.76% of RRF, respectively. IPR 106 is a new alternative for Arabica coffee cultivation without grafting in areas infested by these nematodes.

GENETIC DIVERSITY OF THE ROOT-KNOT NEMATODE, *MELOIDOGYNE INCOGNITA*, BASED ON ITS1 rDNA. **Silva, S.A.¹, R.F. Souza², D.S. Matunaga¹, J.P. Tomaz¹, and A.C.Z. Machado¹.** ¹IAPAR, 86047-902, Londrina, Paraná State, Brazil. ²UEL, 86057-970, Londrina, Paraná State, Brazil.

Root-knot nematodes represent a concern to world agriculture and into this genera *Meloidogyne incognita* highlights due to its wide world distribution and to the great number of host plants amongst wild and cropped species. Several management strategies are used to control this phytonematode, with the genetic control as one of the main strategy, based on the development of resistant cultivars. In this process, one challenge faced by the breeding programs is the resistance outbreaks by some nematode populations. The objective of the present work was to study the genetic diversity of *M. incognita* populations through phylogenetic analysis based on ITS1 rDNA sequences and to establish relationships between this diversity and the parasitism behavior of these populations in their hosts or the α -esterase profiles. For this, ITS1 from pure *M. incognita* populations was sequenced and, using bioinformatics software, phylogenetic trees were generated in order to demonstrate this diversity. Results allowed us to visualize the existence of diversity between *M. incognita* populations, however there was not possible to stablish a relationship between the α -esterase profiles and the phylogenetic groups, and to stablish relationship between populations possessing the capacity of parasitizing resistant cultivars and the phylogenetic groups.

MANAGING CEREAL CYST NEMATODES IN THE WESTERN USA. **Smiley, R.** Emeritus Professor, Oregon State University, Columbia Basin Agricultural Research Center, P.O. Box 370, Pendleton, OR 97801.

Heterodera avenae infests soils in localized regions of at least seven states in the western USA. *Heterodera filipjevi* is also known to occur in three states. These cereal cyst nematodes reduce yields of small grain crops under both rainfed and irrigated conditions. Many growers still do not recognize that one or both of these species are present and are reducing productivity of wheat and barley. Our laboratory developed a species-specific molecular diagnostic tool that has been integrated into services provided by a commercial nematode testing lab. Expanded testing by growers has created an additional awareness that these nematodes must be considered in crop management decisions. Integrated management strategies have previously been focused mostly upon field sanitation, crop rotation, controlling weed grasses, providing adequate fertilizer and, where possible, supplying supplemental irrigation. Two-year rotations of a cereal crop with a non-host broadleaf crop or summer fallow are commonly practiced on infested fields but these short rotations are not adequate for eliminating economic losses. Extended intervals between small grain crops are seldom profitable in this semiarid region, particularly in rainfed agriculture. Chemical and biological nematicides are either not currently available or are not effective or profitable for managing these nematodes, particularly when commodity prices are marginal and when crops are produced on very large rainfed fields. Our search for genetic resistance identified the *Cre1* resistance gene in wheat as being particularly effective against *H. avenae*. Other genes also provided partial protection. However, resistance alone was inadequate because 2nd-stage juveniles invaded roots and caused hypersensitive responses in resistant as well as susceptible plants. The hypersensitive reaction occurred well before the time when resistance was expressed, resulting in a shallow, bushy or knotted root system on resistant as well as susceptible cultivars. In this semiarid region there is little or no precipitation during the summer months and roots of rainfed plants must extract water from deep within the soil profile. Some resistant cultivars on infested soils did not produce competitive grain yields when compared to susceptible cultivars on non-infested soils. Growers were therefore reluctant to plant varieties that are resistant but intolerant. Wheat and barley cultivars exhibiting both resistance and tolerance were therefore required to assure optimal yield performance in the current crop and, at the same time, to reduce the level of risk to a subsequent planting of an intolerant cultivar or crop. Cultivars with dual *H. avenae* resistance plus tolerance traits were identified and are now being advocated for use by growers that have infested fields. But we also identified fields that are infested by both *H. avenae* and *H. filipjevi*. Resistance to one species did not provide resistance to the other species. Resistance plus tolerance to both species must ultimately be pyramided into individual cultivars to achieve a truly sustainable integrated cereal cyst nematode management program in the western USA.

THE SOYBEAN CYST NEMATODE CONTROL IN BRAZIL. **Soares, P.L.M.¹, J.M. dos Santos², and W.P. Dias³.** ¹Professor Assistente, ²Professor Assistente Aposentado, ^{1 e 2}Universidade Estadual Paulista Julio de Mesquita Filho, Faculdade de Ciencias Agrarias e Veterinarias, Departamento de Fitossanidade, Laboratorio de Nematologia, Jaboticabal, SP, Brazil. ²Pesquisador, Embrapa Soja, Caixa Postal 231, Londrina, PR, Brazil.

The soybean cyst nematode (SCN), *Heterodera glycines* Ichinohe, the most aggressive and damaging nematode to soybean, was detected for the first time in the crop of 1991/92 in Brazil, in the cities of Nova Ponte – MG, Chapadao do Sul – MS and Campo Verde – MT. Nowadays it is present in the states of Rio Grande do Sul (RS), Parana (PR), Sao Paulo (SP),

Minas Gerais (MG), Goiás (GO), Mato Grosso do Sul (MS), Mato Grosso (MT), Tocantins (TO), Bahia (BA), Maranhao (MA) and Rondonia (RO). There have been detected 11 races of the nematode in the country (1, 2, 3, 4, 4⁺, 5, 6, 9, 10, 14 and 14⁺). The races 4⁺ and 14⁺ differ from the races 4 and 14, respectively, for the ability to parasitize the North American cultivar Hartwig, which is a standard of resistance for the 16 classic races known in the world. These differences were also verified at a molecular level. The resistance of PI 437654, one of the parentals of Hartwig, to these two races was kept. However, the strong linkage of the resistance alleles with *loco i* (black color of the seed coat) has blocked the transference of the resistance for elite soybean cultivars. A strategy which has worked relatively well is to combine the resistance of PI 437654 with the moderate resistance of PI 88788. The races 4⁺ and 14⁺ were only found in MT, the Brazilian state with the largest area sown with soybean, approximately 10 million of the 30 million cultivated in the country. A survey conducted in 2009 showed that the SCN infests around 1/3 of the area sown with soybean in MT. After MT, MS and GO are the Brazilian states with the largest diversity of races and infested areas with SCN. The nematodes have been spread in the country mainly through vehicles, agricultural machines and seeds that come with soil and, in short distances, through torrents of water and wind (when the soil is revolved). Nevertheless, there are still many areas recognized as been free, even though located in cities considered infested. Prevention is still important and actions to avoid the entrance and dissemination of the nematode should be practiced, but there have been few or almost none actions applied. In areas where the SCN was found, the producer has to live with it, once its elimination is practically impossible, considering the capacity of the cyst to protect the SCN eggs for years in the absence of the host plants. There are no chemical or biological registered products available for the control of SCN, due to their low efficiency. Some measures help to minimize the losses, with highlights for crop rotation/succession with non-host plants and the use of resistant cultivars, when they are available, the combination of both methods is ideal. The planning of rotation is relatively simple, due to the limited range of SCN hosts. However, the adoption of this practice is often limited because of the economic viability of the cultures in certain regions and the preference of the producers in growing soybean, a culture with a higher income. Evaluations on the impact of the plantation of botanic species, non-host crop of *H. glycines* (corn, cotton, sorghum, sunflower, peanut, etc.) in the nematode population, showed that the substitution of soybean for one of those plants, for one crop, reduces the population to a level which allows the return of soybean in the next crop, most of the time. On the other hand, with only one susceptible soybean culture, the population of SCN grows again, necessitating rotation/succession with a non-host plant in the next crop, or sowing a resistant soybean culture. In turn, after two or three years of corn, one can, most of the times, get back to the susceptible soybean for two years, without risk. These recommendations are valid for the conditions where the soil has the pH and base saturation in the recommended levels for the culture, according to the region. Cultivation of non-host crops during the off-season (May to August) did not reduce the nematode population, so the rotation should not be replaced by the succession of cultures. On the other hand, the presence of volunteer soybean plant or host species in the area during off-season contributes to increase the inoculum for the next crop. The use of genetic resistance is more economical for SCN control and better accepted by the producers. However, the sowing of resistant cultivars should not be the only option. Due to the high genetic diversity of the nematode, under selection pressure, new races can be selected. Despite the relatively recent introduction of resistant cultivars, many producers grow resistant soybean in infested areas every crop. There is a high need for SCN resistant soybean cultivars in Brazil, and almost all 90 resistant cultivars currently available are adequate only for races 1 and 3. Even for these two races, there is not an adequate material for all cultivated regions. The other difficulty is that, to combat Asian rust (*Phakopsora pachyrhizi*) and get a second corn, cotton, bean or other culture crop, producer started opting to sow early soybean cultivars, which is not the most SCN resistant cultivar. Pathogen genetic variability has also reduced the lifespan of resistant cultivars. Programs of soybean genetic improvement need to diversify the sources of resistance and producers should avoid monoculture of resistant materials from the same source. The adoption of a rotation scheme which involves non-host cultures, resistant cultivar and susceptible, for example, corn - resistant soybean – susceptible soybean, is the ideal. This might avoid the selection of new races and, thus, the resistance of the cultivar would be prolonged. In the case of MT, it has been common for the producer to repeat every year the sowing of ‘TMG 4182’ soybean, a productive conventional cultivar, with broad resistance to SCN (races 1, 2, 3, 4, 5, 6, 9, 10 and 14). Therefore, new population of nematodes which can beat the resistance of this cultivar will likely soon appear, once these resistant plants are no longer immune and they multiply part of its population.

FROM ENVIRONMENTAL ADAPTATIONS TO INTRASPECIFIC VARIATION IN PHEROMONE BIOSYNTHESIS: AN ESTERASE IS INVOLVED IN THE SYNTHESIS OF COMPLEX SMALL MOLECULES REGULATING DAUER FORMATION IN *PRISTIONCHUS PACIFICUS*. **Sommer, R.J. and J.M. Meyer.** Max Planck Institute for Developmental Biology, Tübingen, Germany.

Animals have evolved abilities to adapt towards changes in their environments. In nematodes, the formation of long-lived, stress-resistant “dauer” larvae represents a key example, which is also known as major survival and dispersal strategy. Small molecules are crucial for regulation of dauer entry and have been shown to function as cues in chemical communication. Recent studies in *Pristionchus pacificus* and comparison to *C. elegans* indicated that small molecule structure and function evolve rapidly. *P. pacificus* contains small-molecules with building blocks from diverse primary metabolic pathways, such as ubas#1 that consists of an ascaroside to which an oxygenated, second ascaroside is attached by an ester bound. Furthermore, a

3-ureido isobutyrate moiety is attached to carbon 4 of the ascaroside resulting in a complex structure unknown from *C. elegans* small-molecules. However, very little is known about the enzymes and pathways involved in the synthesis of such complex small-molecules. We will highlight our approaches that aim to observe adaptations to extreme environments such as those on La Réunion Island and our attempts to associate these adaptations to molecular and biochemical functions. We report our studies on intra-species variation in small molecules production in *P. pacificus* on La Réunion Island. In the last 5 years, we extensively sampled a nematode population by collecting more than 300 local strains, which were subsequently characterized through a combination of genomic (RAD-sequencing) and secretomic (HPLC/MS/MS) approaches. Performing genome-wide association studies (GWAS), we identified a single candidate region consisting of an operon of three esterase genes. Re-sequencing of *ubas#1*-deficient wild isolates indicated a deletion of one of these esterase genes. CRISPR-Cas9-induced mutation of this gene in the *P. pacificus* reference strain converted this strain from *ubas#1*-positive to *ubas#1*-negative strain. Thus, our study has identified an esterase involved in the synthesis of complex small-molecules. We will place our findings in the broader context of adaptations to extreme environments and will highlight the power of the *P. pacificus* system from integrative studies that link molecular investigations of lab-based studies to ecology, population genetics and phylogeny.

SOIL FREE-LIVING NEMATODES AS BIO-INDICATORS FOR ASSAYING THE INVASIVE ALIEN PLANT EFFECT IN A COASTAL DUNE ECOSYSTEM. **Steinberger, Y.¹ and N. Fitoussi¹.** ¹ The Mina and Everard Goodman Faculty of Life Sciences, Bar-Ilan University, Ramat-Gan 5290002, Israel.

Invasive plant species are thought to threaten native above and below ground biota biodiversity. The two main major ecological consequences known are: (1) displacing native plant and animal species and (2) changing physical habitat's properties (i.e. transformers). The threat on native plants is of high severity as invasion of species may be considered to be one of the major threats to the below ground biota after habitat destruction. Such change is supposed to affect soil biota community / nutrient cycling affecting plant-soil feedback loops that reinforce the success of invasive species. In the present study we investigated whether and how the *Heterotheca subaxillaris* plant that was brought to Israel in 1975 from Central America in order to stop sand dune movement along the sea shore sand dunes habitats. *H. subaxillaris* had become an invasive plant that differs from the native species *Artemisia monosperma* in its functional traits and effects on soil biota. A field study was initiated in order to establish how *H. subaxillaris* invasion plant differs from *A. monosperma* the most widespread native species in its effects on soil free living nematode community, trophic group composition and diversity. Soil samples (n = 24) were collected from the upper (0-10 cm) soil layer under *H. subaxillaris* (invasive perennial shrub) and *Artemisia monosperma* (native perennial shrub) at two different sites in the sea-sand ecosystem during the winter and summer seasons. Molecular analysis using the 18S rRNA gene for species determination, along with the traditional method had showed that the nematode communities, trophic diversity, and taxon composition had found to be affected by the invasive plant in comparison to the native plant cover.

IMPROVING SOIL HEALTH AND ENHANCING SUPPRESSIVE SERVICES IN SUGARCANE SOILS. **Stirling, G.¹ and D.E. Walter².** ¹ Biological Crop Protection Pty. Ltd, Moggill, QLD, 4070, Australia. ² University of the Sunshine Coast, Sippy Downs, QLD, 4556, Australia.

Sugarcane was first planted in Australia during the 1880s and within a few years, soil health problems were apparent. Technical papers published between 1900 and 1938 indicated that a 'sick soil' syndrome was widespread and seemed to be associated with continuous monoculture and depletion of soil organic matter. When mechanical harvesting was introduced in the 1960s, soil health problems worsened because the soil was compacted due to mismatched wheel and row spacings. Productivity (sugar yield per harvested hectare) reached a plateau between 1970 and 1990 and this prompted the industry to establish a multi-disciplinary research team (the Sugar Yield Decline Joint Venture) to find solutions to the yield decline problem. Following 10 years of research, a new farming system was developed that incorporated the following practices: 1) permanent raised beds maintained through traffic control; 2) a direct-drilled legume grown in rotation after the sugarcane was terminated with herbicide; 3) double-disc openers used to replant sugarcane with minimal soil disturbance; and 4) sugarcane harvested green with a cover of plant residues (the trash blanket) permanently maintained on the soil surface. Recent research has focused on the benefits obtained from this farming system. Surveys have shown that root biomass is greatest in the upper part of the soil profile where soil carbon levels are highest (i.e. the soil immediately below the trash blanket). These surface roots are also quite healthy, harbouring 55-70% fewer *Pratylenchus zaei* and *Meloidogyne* spp. than roots further down the profile. Pot experiments in which sugarcane was grown in soils collected from different depths confirmed the observations made in the field. Plants grew much better in surface soils, with aboveground biomass 35-100% higher than in soils from depths of 2.5-10 cm. The soil's carbon status also had a huge impact on populations of *P. zaei* in roots. In sandy loam soils, for example, an increase in soil carbon levels from 1% to 1.5% resulted in numbers of lesion nematodes being reduced by about 80% (from more than 21,000 nematodes/g dry weight of root to about 4,000). Soil microarthropods appear to be contributing to this effect, as more than 30 predatory species have been found in well-managed sugarcane soils. Some mites fed voraciously on nematodes in the laboratory and an initial experiment in pots showed that a species of *Protogamasellus*

reduced populations of *Tylenchorhynchus annulatus*, *P. zaeae* and microbivorous nematodes by 99, 70 and 70%, respectively. A third tier of research aims to determine whether *Pasteuria*, a bacterial parasite of nematodes, is having an impact on nematode populations. *Pasteuria* endospores have been seen on *Meloidogyne javanica*, *Helicotylenchus dihystra*, *P. zaeae* and *T. annulatus* and a number of sites with relatively high infestation levels have been identified. The effects of natural and introduced infestations of *Pasteuria* are currently being studied.

MAPPING THE *GLOBODERA PALLIDA* RESISTANCE GENE *H2* IN POTATO USING WHOLE GENOME ENRICHMENT. **Strachan, S., K. Baker, I. Hein, G. Bryan, and V. Blok.** The James Hutton Institute, Dundee, Scotland, DD2 5DA.

Plant parasitic nematodes (PPN) of the genera *Meloidogyne*, *Heterodera*, and *Globodera* are the most economically important PPNs. These nematodes invade host plants roots where they establish a multinucleate feeding site. These feeding sites, commonly known as syncytium, are the nematodes sole source of nutrients, and it is this structure which causes the host plant most damage by diverting nutrients away from the root cells toward the nematode. The nematode feeds at this syncytium for several weeks before moulting to adulthood and completing its lifecycle. The potato parasitic nematodes *Globodera rostochiensis* and *G. pallida* encapsulate their fertilised eggs in a tough outer cyst which allows them to persist in the soil for decades, while still remaining viable, making control by crop rotation a lengthy and not always successful process. Control with nematicides is diminishing due to legislation (European Union directive (99/414/EEC)) prohibiting their use due to environmental and human health concerns and because of their limited effectiveness. Exploiting the natural resistance present in wild potato is a highly effective method to combat these pests. The *H1* gene from *Solanum tuberosum* ssp. *andigena* CPC 1673 confers almost complete resistance to *G. rostochiensis* pathotypes Ro1 and Ro4, and its integration into commercial cultivars has been effective in reducing the threat from *G. rostochiensis* in Britain. This however has led to a shift in species prevalence, making *G. pallida* the UK's main PCN problem. *G. pallida* populations in the UK are more genetically diverse than *G. rostochiensis*, and as a result no single resistance gene is likely to be both effective and durable for the pathotypes (Pa1, Pa2/3) present in British potato fields. Some success has been found with major quantitative trait loci (QTL) such as *GpaV* and *GpaIV^{sdg}* from *S. tuberosum* ssp. *vernei*, and *Solanum tuberosum* ssp. *andigena* CPC 2802, respectively. One strategy that is now being employed is to combine (pyramid) several resistance genes, which are effective against different pathotypes into one cultivar, to give a high level of broad spectrum resistance. The major resistance gene *H2* from *S. multidissectum* confers a high level of resistance against the Pa1 pathotype of *G. pallida* and partial resistance to the Pa2/3 pathotype. Its position in the potato genome remains unmapped, and here we outline the method used to determine its location.

AGAMERMIS (NEMATODA: MERMITHIDAE) INFECTION IN SOUTH CAROLINA AGRICULTURAL PESTS. **Stubbins, F.¹, P. Agudelo², F.P.F. Reay-Jones³, and J.K. Greene¹.** ¹Clemson University, Department of Plant and Environmental Sciences, Edisto Research and Education Center, 64 Research Rd, Blackville, SC 29817, ²Clemson University, Department of Plant and Environmental Sciences, Plant Nematology Laboratory, Clemson, SC 29634, ³Clemson University, Department of Plant and Environmental Sciences, Pee Dee Research and Education Center, 2200 Pocket Rd, Florence, SC 29506.

Native and invasive stink bugs (Hemiptera: Pentatomidae) and the closely related invasive *Megacopta cribraria* (Hemiptera: Plataspidae) are agricultural pests in the southeastern United States. Natural enemies across different phyla parasitize these species and contribute to pest population regulation. We specifically investigated Nematoda infections in pentatomid and plataspid pests in one soybean field in South Carolina in 2015. Nematodes were identified through molecular and morphological methods and assigned to family Mermithidae, genus *Agamermis*. This study reports mermithid nematode infection in immature *M. cribraria* for the first time and provides the first host record for the stink bugs: *Chinavia hilaris*, *Euschistus servus*, and another *Euschistus* species, and a grasshopper (Orthoptera: Acrididae) in South Carolina. The same *Agamermis* species infected all hosts. The broad host range and prevalence suggests that *Agamermis* may be an important contributor to natural hemipteran pest mortality. Further work is needed to assess the impact of infection on populations over a broader range of agricultural fields and geographic localities.

GLOBODERA SPECIES: CURRENT SYSTEMATICS AND PHYLOGEOGRAPHY. **Subbotin, S.A.¹, R. Knoetze², I. Cid Del Prado Vera³.** ¹Plant Pest Diagnostics Center, California Department of Food and Agriculture, 3294 Meadowview Road, Sacramento, CA 95832-1448, USA; ²Directorate Inspection Services, Department of Agriculture, Forestry and Fisheries, Private Bag X5015, Stellenbosch 7599, South Africa; ³Colegio de Postgraduados, Montecillo 56230, Mexico.

The genus *Globodera* presently contains twelve valid and five still undescribed species. Three species, *G. rostochiensis*, *G. pallida* and *G. ellingtonae* are named as potato cyst nematodes and cause significant economic losses on potatoes around the world. The phylogenetic analysis of rRNA genes revealed two distinct clades within the genus *Globodera*, which correspond to geographical origins and plant-host associations. The hypothesis of an 'out of Gondwana' origin of the genus *Globodera* with subsequent dispersal of the species of this genus to Europe, North America, Asia and Oceania found the support from the analysis of the ITS rRNA gene sequences. Several centers of *Globodera* speciation and its associations with mountain

regions of the world are hypothesized. Using mtDNA genes it has been shown that *G. pallida* and *G. rostochiensis* originated from Andes mountains, South America and *G. pallida* presently distributed in Europe and North America derived from a restricted location in south of Peru. The diagnostics of *Globodera* species is mainly based on several morphometric and morphological characters, including the number of cuticular ridges between anus and fenestra, Granek's ratio, stylet, tail and hyaline region of tail lengths and shapes of stylet knobs. The taxonomic status and relationships of *G. mexicana* with other species are discussed. Molecular techniques including PCR-ITS-RFLP, *conventional and Real Time PCR* with specific primers, sequencing of ITS ribosomal RNA gene are currently used for routine diagnostics of potato cyst nematodes in many laboratories. Restriction of PCR-ITS-rRNA amplicons by five enzymes distinguishes several species of *Globodera* from each other. However, there are some needs for expanded practical testing of all these methods across a range of *Globodera* species in order to confirm the reliability of presently proposed diagnostic approaches.

EVALUATION OF SHIELDING EFFICACY OF BOVINE SERUM ALBUMIN AND POLY ETHYLENE AMINE ON GRAPHENE OXIDE BY USING THE NEMATODE MODEL *CAENORHABDITIS ELEGANS*. **Subramani, S.¹, S. Govindan², P. Sundararaj², S.L. Hafez³, P. Nagamony¹ and N. Sundararaj⁴.** ¹Department of Nanoscience and Technology, Bharathiar University, Coimbatore 641046, India ²Department of Zoology, Bharathiar University, Coimbatore 641046, India ³University of Idaho Parma REC, 29603 U of I Lane, Parma, ID 83660, USA ⁴Kumaraguru College of Technology, Coimbatore, India.

Graphene oxide (GO) has been extensively used in nanomedicine especially in; gene and drug delivery, stem cell differentiation, cell growth control, cancer treatment, enzyme immobilization and also in biosensors. However, the lowered stability in the blood stream and toxic effects to the human system, has limited the usage of GO. In the present work, we have investigated the shielding efficacy of GO coated polyethylene amine (PEI) or bovine serum albumin (BSA) by using the nematode model *C.elegans*. The GO was synthesized by using modified Hummer's method. These prepared GO NPs were coated with either PEI or BSA and mixed with *Escherichia coli* OP50 and fed to the L2 stage of *C.elegans* maintained on 0.8% agar. Enhanced dark-field microscopy was used to identify the localized GO in the intestinal tract of the *C.elegans* after 24h treatment. Acute toxicity was measured in bare GO NPs treated with *C.elegans* compared with PEI or BSA coated NPs. BSA coated GO NPs had a reduced toxicity to *C.elegans* when compared to PEI coated NPs. This study clearly confirms that the protein surface engineering of GO NPs plays a vital role in reducing particle toxicity in the biological environment.

WHITE TIP NEMATODE FINDINGS IN ARKANSAS AND LOUISIANA RICE. **Sullivan, Katie¹, D.M. Xavier-Mis², R.J. Bateman¹, C. Overstreet², and T.L. Kirkpatrick¹.** ¹Plant Pathology Dept., University of Arkansas, Hope, AR 71801, ²LSU AgCenter, Plant Pathology and Crop Physiology Dept., Baton Rouge, LA 70803.

Arkansas and Louisiana are in the top five rice producing states in the United States (first and third, respectively). About half of the U.S. rice crop is exported each year to Mexico, Central America, Northeast Asia, the Caribbean, and the Middle East. A requirement for exporting rice into some countries is that the rice seed be tested and certified as free from the white tip nematode (*Aphelenchoides besseyi*). The Louisiana Nematode Advisory Service (LNAS) detected white tip nematode in a rough rice sample from a barge in late 2014 to be exported to Turkey. During 2015, *A. besseyi* was detected in 37 of 98 samples of rice seed assayed for regulatory purposes. Similarly, the Arkansas Nematode Diagnostic Laboratory (ANDL) detected *A. besseyi* in 37 of 179 samples that were submitted in 2015 by regulatory agencies. Prior to 2015, no white tip nematodes were detected in regulatory samples in the LNAS and only one positive sample was reported in 2011 by the ANDL. Surveys of commercial rice fields conducted in 2010 and 2015 in Arkansas indicate that *A. besseyi* was present in four of 135 fields in 2010 and seven of 74 fields in 2015. Population densities of white tip nematode in these samples in 2010 were low with < 10 per 25 grams of seed, while in the 2015 survey samples ranged from 1 to 409 nematodes per 25 grams of rice seed. Although white tip nematode has been considered to be a minor pest for many years in the U.S., these findings suggest that this ancient pest is still present and has the potential to impact rice production.

A MULTIPLEX REAL-TIME PCR ASSAY FOR SIMULTANEOUS DETECTION AND DIFFERENTIATION OF *DITYLENCHUS DIPSACI*, *D. GIGAS*, AND *D. WEISCHERI*. **Sun, F.¹, N. Henry¹, S. Craig¹, G. Bilodeau,¹ Q. Yu,² and P. Castillo³.** ¹ Canadian Food Inspection Agency, 3851 Fallowfield Road, Ottawa, ON, Canada, K2H 8P9, ² Ottawa Research and Development Center, Agriculture and Agri-Food Canada, Ottawa, ON K1A 0C6, Canada, ³ Departamento de Proteccion de Cultivos, Instituto de Agricultura Sostenible (IAS), Consejo Superior de Investigaciones Científicas (CSIC), Avda. Menendez Pidal s/n, 14004 Cordoba, Spain

The stem nematodes, *Ditylenchus dipsaci*, *D. gigas*, and *D. weischeri*, are closely related species that were previously considered as different races of *D. dipsaci* species complex. The fourth-stage (J4) of these species can survive many years of desiccation in plant tissue, seed, and soil in a state of cryptobiosis. Dry seeds of the host plants carrying these pests are an important means of dissemination from one region to another. *Ditylenchus dipsaci* and *D. gigas* are regulated quarantine pests in many countries and subject to inspection during international trade of seed and grain, whereas *D. weischeri*, a parasite of creeping thistle or Canada thistle *Cirsium arvense*, causes little damage to agricultural crops. However, the presence of *D.*

weischeri in field pea grain contaminated with *D. weischeri*-infested thistle seed can cause confusion due to the morphological similarity of J4 stages among these three species. A TaqMan-based multiplex real-time PCR assay was developed in this study to simultaneously differentiate *D. dipsaci*, *D. gigas*, and *D. weischeri*. Primers and species-specific probes, targeting the heat shock protein (*hsp90*) gene, successfully detected and identified single nematode of several populations of the *Ditylenchus* species, alone or in mixture. This rapid, sensitive, and species-specific quantitative PCR assay presents a reliable tool for regulatory response and management programs.

CHEMICAL SIGNALS MEDIATE TRIPARTITE INTERACTIONS BETWEEN PINEWOOD NEMATODE, ITS VECTOR BEETLE AND ASSOCIATED FUNGI Sun, J.¹ and L.L. Zhao¹. ¹State Key Laboratory of Integrated Management of Pest Insects and Rodents, Institute of Zoology, Chinese Academy of Sciences, Beijing 100101, P. R. China

Pine wilt disease (PWD) is perhaps the most serious threat to pine forests worldwide. The causative agent of PWD, the pinewood nematode (PWN), engages in a symbiotic partnership with its insect vector, *Monochamus alternatus*, as well as associated bacteria and ophiostomatoid fungi, in order to successfully infect and kill its host pine tree. The interspecific chemical communications between PWN and its associated partners, and their implications in enhancing pathogenicity and invasiveness of PWN are reviewed in this presentation. The work presented here from our laboratory demonstrates that associated fungi played a role in the prevalence and spread of PWN in China. In particular, the nematode produced greater numbers of offspring, developed faster, and produced a higher proportion of progeny in the presence of one native blue-stain fungus *Sporothrix* sp.1. Diacetone alcohol from host pine xylem inoculated with *Sporothrix* sp.1 induced *B. xylophilus* to produce greater numbers of offspring. Its presence also significantly increased the growth of *M. alternatus*. Furthermore, we show that L_{IV} formation in *B. xylophilus* is induced by C16 and C18 fatty acid ethyl esters (FAEEs), which are produced abundantly on the body surface of the vector beetle specifically during eclosion. The L_{IV} can then enter the tracheal system of the newly eclosed adult beetle for dispersal to a new pine tree host. Our work also showed that C16 and C18 FAEEs modulate the insulin/IGF-1 signaling pathway and the downstream dafachronic acid-DAF-12 endocrine pathway to induce L_{IV} formation in *B. xylophilus*, which is similar to dauer formation in the free-living nematode *Caenorhabditis elegans*. This suggests a mechanism by which dispersal L_{IV} formation in *B. xylophilus* is specifically coordinated with the life cycle of its vector beetle. Knowledge of the chemical signals and understanding of those interactions could be used to interfere with the dispersal of this plant-parasitic nematode.

NEMATODE DEFENSINS IN CONTEXT: THE CYSTEINE-STABILIZED ALPHA-BETA (CS- $\alpha\beta$) SUPERFAMILY OF DEFENSIVE PEPTIDES. Tarr, D.E.K. Department of Microbiology and Immunology, Arizona College of Osteopathic Medicine, Midwestern University, Glendale, AZ 85308.

The term “invertebrate defensin” has become increasingly vague as more cysteine-rich antimicrobial peptides have been identified, annotated, and to a lesser extent, characterized. Invertebrate defensins belong to the cysteine-stabilized alpha-beta (CS- $\alpha\beta$), also known as the scorpion toxin-like, superfamily. Unfortunately, there are peptides that belong to this superfamily that have been given other names, and are indistinguishable from those that are officially called “defensins.” In nematodes, these are the antibacterial factors (ABFs) and cremycins, as well as many sequences that have been identified but not named. There are no established criteria for designating a sequence as a “defensin.” In addition, there are other groups of defensins that are not evolutionarily related to this group, which complicates phylogenetic analyses and discussions of invertebrate defensins. I have developed a ten-cysteine reference array that aligns structurally significant cysteines of sequences in this superfamily to clarify their characteristic features. Nematode sequences identified thus far should be considered defensins—in fact, nomenclature for these peptides that is currently in use is somewhat misleading and not sufficiently supported by experimental evidence.

RESPUESTAS DE DEFENSA EN DIFERENTES ESTRATOS DEL SISTEMA RADICAL DE PLANTAS DE CHILE INOCULADAS CON *NACOBBUS ABERRANS* Y/O *PHYTOPHTHORA CAPSICI*. Téllez-Álvarez, D.¹, L.C. Pérez-Viveros¹, N. López-Martínez¹, and E. Zavaleta-Mejía². ¹Departamento de Fitotecnia, Universidad Autónoma Chapingo. Texcoco C.P. 56230, México, ²Instituto de Fitosanidad, Colegio de Postgraduados, Montecillo, Edo. De México, C.P. 56230, México.

El genotipo de chile CM334 (*Capsicum annuum* L.) es resistente a *Phytophthora capsici* Leonian, pero el nemátodo falso agallador *Nacobbus aberrans* (Thorne, 1935) Thorne & Allen, 1944 si está presente inhibe su resistencia. Conocer los cambios metabólicos inducidos por *N. aberrans* pueden ayudar a comprender el fenómeno de pérdida o inhibición de la resistencia. Por lo tanto, en éste trabajo se evaluó la actividad de las enzimas peroxidasas (POX) y fenilalanina amonio liasa (PAL), así como el contenido de fenoles solubles totales (FST), en dos estratos del sistema radical (ápices y sistema radical desprovisto de ápices) de plantas de chile CM334 inoculadas con *N. aberrans*, solo o en combinación con *P. capsici*. La inoculación con el oomiceto se realizó a los 21 días después de haber inoculado a *N. aberrans*. A las 6 horas después de inoculación con *P. capsici*, en promedio, el sistema radical sin ápices presentó valores de actividad de peroxidasas que fueron el doble de las correspondientes a los ápices de la raíz. En cambio, la mayor actividad de PAL y FST se registró en los ápices

del sistema radical (2.7 y 0.3 veces más, respectivamente). La mayor actividad de las peroxidasas del sistema radical sin ápices fue registrado en las plantas inoculadas con *P. capsici* (13.7 μMoles de tetraguaiacol mg^{-1} de proteína min^{-1}) y las diferencias con los demás tratamientos fueron estadísticamente diferentes ($P \leq 0.05$). En cambio, en el ápice radical de las plantas inoculadas con ambos patógenos la actividad de las peroxidasas fue significativamente mayor a las de las plantas que conformaron los demás tratamientos. En ambos estratos de la raíz la mayor actividad de la PAL (38.7 nMoles de ácido *trans*-cinámico μg^{-1} de proteína min^{-1}) y de FST (11.3 mg de ácido tánico g^{-1} de residuo seco) se registró en las plantas inoculadas sólo con *P. capsici*, mientras que, en los tratamientos que involucraron a *N. aberrans* (solo o en combinación con *P. capsici*) se presentaron niveles menores y similares al testigo ($P \leq 0.05$). Éstos resultados proporcionan algunos avances relacionados con la interacción entre dos patógenos diferentes de *C. annuum* y su efecto sinérgico en la resistencia del hospedero.

SURVEY SAYS: NO SOYBEAN CYST NEMATODE, *HETERODERA GLYCINES* ICHINOHE IN MANITOBA. Tenuta, M., M. Madani, F. Peirera, and A. Hajhassani. Department of Soil Science, University of Manitoba, Winnipeg, MB, Canada R3T 2N2.

This report details results of a survey of commercial soybean (*Glycine max*) fields for *Heterodera glycines* Ichinohe, 1952, (Soybean Cyst Nematode; SCN), conducted in 2014-2015 in the Province of Manitoba, Canada. *Heterodera glycines* is recognized as the major pest of soybean worldwide. With respect to soybean cultivation for Manitoba farmers, early detection and precise identification is of significant importance. Soybean cyst nematode has rapidly moved northward in the mid US states. It is now present in some of the counties of North Dakota and Minnesota bordering Manitoba. It is only time until it is Manitoba, if not already. Recently, the Canadian Food Inspection Agency has declassified SCN as a regulated pest in Canada. This means to farmers that surveys for the nematode are no longer done by the agency. In the current study, 28 fields were sampled for a total of 205 soil samples analyzed for the presence of SCN. Nematode cysts were recovered from 32 soil samples. The samples yielded one to a few cysts each, with the majority being empty and broken. Further, most cysts were round and not lemon-shaped, the later a possible indicator of SCN. Further, most cyst cone tops were circumfenestrate rather than bifenestrate, the later possibly indicating SCN. Only six samples yielded DNA suitable for PCR analysis and these were all negative for SCN. With the current and past survey conducted by the Soil Ecology Laboratory, a total of 76 commercial soybean fields in Manitoba have been sampled and are negative for the presence of SCN. Most fields in Manitoba have a history of three or less crops of soybean. That soybean acreage has recently surpassed 1,300,000 acres/year in Manitoba, it may still be a few more years until SCN populations are detected. Further, because SCN is near the North Dakota and Minnesota border with Manitoba, it is recommended surveys be conducted every two to three years.

PRE-HATCH DEVELOPMENT OF *HETERODERA GLYCINES* ENCYSTED AND EGG-MASS EGGS IN DIFFERENT HATCH STIMULANTS. Thapa, S., J.A. Patel and N.E. Schroeder. Dept. of Crop Sciences, University of Illinois, Urbana, IL, 61801.

Soybean cyst nematode (SCN)-*Heterodera glycines* is a devastating pest in most soybean growing countries. SCN females produce eggs within the female cyst (encysted eggs) and in a gelatinous matrix (egg-mass eggs). Dormant eggs may remain viable for up to 11 years. The hatching behaviors of SCN are coordinated to exploit the availability of suitable plant hosts and to avoid exposure to unfavorable environmental conditions. Data suggest different hatching behaviors in different hatch stimulants. It has been reported that egg-mass eggs hatch more rapidly than encysted eggs. Management techniques based on hatching behaviors may be designed to control SCN. The main objectives of this study were: i) to create a detailed time-line of pre-hatch development of SCN; ii) to assess if this time-line differs between encysted and egg-mass eggs as well as between different hatch stimulants; and iii) to assess the neuroanatomy of pre-hatch J2. We began all embryonic time-series analyses at the two-celled embryo using the hanging drop method. Two-celled eggs were the result of transverse cleavage producing cells of approximately the same size. The second transverse cleavage was between 2-4 h and the third cleavage was between 3-7 h. Rapid longitudinal and transverse cell divisions led to the formation of multi-celled stages. A single layer of cells surrounding the internal cells were observed in the blastula stage between 45-53 h and gastrulation was observed between 69-76 h. The embryo began moving following the development of the tadpole stage between 96-106 h and soon after became worm shaped. A coiled J1 was formed between 104-118 h. Cells differentiated into two zones of dissimilar densities; the light zone become the esophagus and the dark zone became the intestine. After molting, the stylet was observed in the J2 pre-hatch stage between 159-180 h. The fully formed unhatched J2 showed continuous movement interrupted by short periods of quiescence. It continued circulating within the eggshell and eventually made a slit near the pole with the stylet for eclosion. There was substantial variation in the developmental time-line within the eggs from cysts and from egg-masses. The pre-hatch developmental time-line between encysted and egg-mass eggs was not significantly different. Similarly, no significant differences in the developmental timeline were observed between water and either hatching stimulant. This suggests that hatching stimulants do not affect the embryogenesis process but influence the hatching decision once the J2 has formed. Previous research showed that the hatched J2 contains 66 nuclei within the ventral nerve cord. We examined the ventral nerve cord of pre-hatch animals using a nuclear stain. The ventral nerve cord of early and late pre-hatched J2 on average had 57 and 61 neurons respectively, suggesting that the nervous system is not completed until after the first molt.

AN INTEGRATED APPROACH TO IMPROVING CORN PLANT HEALTH IN *MELOIDOGYNE INCOGNITA* INFESTED FIELDS WITH NEMATICIDES, PLANT GROWTH REGULATORS, AND STARTER FERTILIZERS. **Till, S. and K.S. Lawrence.** Dept. of Entomology and Plant Pathology, Auburn University, AL, 36849.

Meloidogyne incognita, the southern root knot nematode, is responsible for significant yield losses across the southern portion of the United States and losses as high as 30% can occur in field corn, *Zea mays*. We hypothesize that adding additional inputs (starter fertilizers and plant growth regulators) at planting along with nematicides can provide for a complete management system of both *M. incognita* and the corn plant by improving plant health at the same time as suppressing nematode population densities. Each input was evaluated separately in a greenhouse setting. Data was analyzed with SAS 9.4 using PROC GLIMMIX and LS-means were compared using Dunnett's method with significant level of $\alpha \leq 0.1$. In the nematicide trial, growth parameters (shoot/root fresh weights and biomass) were greater in the untreated control ($P \leq 0.1$) than with Terbufos and Clothianidin/*Bacillus firmus* + Fluopyram/Imidacloprid nematicides at 14 days after planting (DAP). However, at 45 DAP the untreated control's growth parameters were all lower ($P \leq 0.1$) than the nematicide treatments. Terbufos, Fluopyram/Imidacloprid, and Clothianidin/*Bacillus firmus* + Fluopyram/Imidacloprid all reduced root knot egg production ($P \leq 0.1$), and Terbufos increased biomass ($P \leq 0.1$) relative to the untreated control. In the plant growth regulator trial, Ascend (0.090% cytokinin: 0.030% gibberellic acid: 0.045% indolebutyric acid) was the product selected and the efficacy of single to multiple applications were evaluated. At 45 DAP, the in-furrow application (365 ml/ha rate) improved plant growth parameters (shoot/root fresh weight and biomass) ($P \leq 0.1$) relative to the untreated control, and was similar to the untreated control in eggs per gram of root. The triple combination (in-furrow + foliar + seed treatment) supported increased numbers of root knot eggs per gram of root ($P \leq 0.1$) relative to the untreated control. The starter fertilizer treatments all increased plant biomass ($P \leq 0.1$) relative to the untreated control at 45 DAP with the exception of Micro-500 and Neptune's Harvest. From the greenhouse tests, we selected nematicides Terbufos and Fluopyram, the plant growth regulator's in-furrow application, and the combination of starter fertilizers (Pro-Germinator + Sure-K + Micro 500) to be further evaluated in field and microplot settings to determine yield effects.

THE EFFECT OF NIMITZ® (FLUENSULFONE) ALONE AND IN COMBINATION WITH PARTNER PRODUCTS AGAINST *N. ABERRANS* INFECTING GREENHOUSE TOMATO. **Trejo-Díaz, R.¹, J.A. Vázquez-Hernández¹, A.J. Cabrera-Hidalgo¹, N. Marban-Mendoza¹, and F. Chaverri².** ¹Universidad Autónoma Chapingo, Carr. México-Texcoco km 38.5, Chapingo, Edo. de México, C.P. 56230. ²Instituto Regional de Estudios en Sustancias Tóxicas. Universidad Nacional, 86-3000. Heredia, Costa Rica.

Fluensulfone (Nimitz) is a non-fumigant nematicide of the fluoroalkenyl thioether group, and has significantly lower environmental impact and toxicity to non-target insects and mammals compared to many of the currently available chemical controls e.g. methyl bromide, organophosphates and carbamates. The latter pesticides have unacceptable levels of toxicity to non-target organisms and are being withdrawn from use because of their negative impact on human health and the environment. Nimitz® (Fluensulfone) alone (2.25 L·ha⁻¹) and in combination with partner products (1.75 and 2.0 L·ha⁻¹): Nematrol plus® (Chitosan and plant extracts), Nemover® (extract of pine, oregano and castor) and Ditera DF® (*Myrothecium verrucaria*) were assayed in greenhouse trials against *Nacobbus aberrans* affecting tomato (*Solanum lycopersicon* L.). Nimitz was applied 10 days before transplanting and the other products three times at 15 day intervals starting at 90 days after transplanting (DAT). All treatments were distributed under a randomized completely block design with three replications. At 165 DAT, nematode levels, root galling, control effect [what is control effect?] and yield were assessed. All treatments that included Nimitz were significant ($P = 0.0001$). The lowest nematode levels were found in the Nimitz plus Ditera treatment (up to 75% reduction compared to untreated control). Tomato plants treated with Oxamyl were initially protected, but root galling increased up to 88% at 120 days after treatment. Root galling was much lower in the Nimitz (2 L·ha⁻¹) plus Nematrol plus® treatment. Nimitz (2 L·ha⁻¹) treatments on average doubled the fruit yield compared to the untreated control.

INTEGRATED MANAGEMENT OF *HETERODERA GLYCINES* IN THE MIDWESTERN UNITED STATES. **Tylka, G.L.** Department of Plant Pathology & Microbiology, Iowa State University, Ames, IA 50011.

More than 75 percent of the soybean crop in the United States is grown in the Midwest. And the soybean cyst nematode (SCN), *Heterodera glycines*, is one of the most widespread and damaging soybean pathogens in the region. Management consists primarily through growing nonhost crops and SCN-resistant soybean cultivars and using nematode-protectant seed treatments on soybeans. Growing nonhost crops reduces SCN population densities, and the decrease may be as much as 50 percent in a single year of corn following soybean. But declines in SCN population densities diminish greatly after one or two successive years of corn. Also, corn is the only crop other than soybean that is widely grown in the Midwest, and continuous corn production can be problematic and unprofitable. Consequently, it is not feasible to manage SCN simply by growing nonhost corn in rotation with soybean. There are hundreds of commercially available soybean cultivars described as resistant to SCN. Many cultivars can produce high yields and prevent increases in SCN population densities throughout the growing season. Resistant soybean cultivars often yield five to forty percent more than susceptible cultivars in SCN-infested fields.

And end-of-season SCN population densities under SCN-resistant soybean cultivars typically are only one quarter to one third of SCN densities produced under susceptible cultivars. Unfortunately, almost all commercially available SCN-resistant soybean cultivars in the Midwest since 1990 have had resistance genes from a breeding line named PI 88788. Prolonged, widespread use of PI 88788 SCN resistance has selected for SCN populations with increased reproduction on resistant soybean cultivars. This shift in virulence of SCN populations has resulted in reduced yields of resistant cultivars and increased end-of-season nematode population densities. Nematode-protectant seed treatments for SCN management became available in the mid 2000s and currently include Avicta[®], Clariva[™], Ileva[®], and Votivo[®]. The seed treatments are used on SCN-resistant soybean cultivars. The effects of the seed treatments in field experiments in the Midwest have been variable. In small-plot field experiments in Iowa over 27 location-years in 2014 and 2015, seed treatments have had no effect on soybean yields or SCN population densities, have increased soybean yields but had no effect on season-long SCN reproduction, or have not affected yields but decreased season-long SCN reproduction. To date, seed treatments have not increased soybean yields and decreased SCN reproduction in the same experiment. These results underscore the variable nature of the effects of nematode-protectant seed treatments on SCN and soybeans. In summary, there are multiple strategies that soybean farmers can use to manage SCN. But few SCN nonhost crops are grown in the Midwest other than corn, the effectiveness of soybean cultivars with PI 88788 resistance is decreasing, and the effects of nematode-protectant seed treatments seem inconsistent. Improved and additional, new SCN management options are needed in order for soybean production to continue to be profitable in SCN-infested fields in the Midwestern United States.

IDENTIFICATION AND REPRODUCTION OF PIN NEMATODES ON FIELD PEA (*PISUM SATIVUM*) IN NORTH DAKOTA. Upadhaya, A.¹, G.P. Yan¹, A. Plaisance¹, J. Pasche¹, and K. McPhee². ¹North Dakota State University, Department of Plant Pathology, Fargo, ND 58108, ²NDSU, Department of Plant Sciences, Fargo, ND 58108.

Pin nematodes (PN), migratory ectoparasites, were found to be the major plant-parasitic nematodes in pea fields in North Dakota. In 2015, 91 soil samples were collected from 31 fields in 9 counties. PN were present in 60% of the samples with highest density of 21,500 per kg of soil, followed by spiral (22%), stunt (21%), dagger (8%), root-lesion (2%), and stubby root (1%) nematodes. More than 97% of the PN populations in the fields were fourth-stage juveniles (J-4) with no stylet present, and less than 3% of the populations were stylet-bearing, feeding adults. Soil samples from three fields with similar soil texture (sand: 49.0 to 56.5%; clay: 15.0 to 20.0%; silt: 25.5 to 33.5%) were used for the PN reproduction study. Reproduction of these PN were evaluated at four initial population levels (3000, 5000, 6000, and 13,000 nematodes/kg soil) using three cultivars of field pea (Columbian, Aragorn, and Cooper). Reproduction rate was the highest at 6,000 nematodes/kg soil. PN (with a stylet plus without a stylet) was able to reproduce in all the cultivars with overall mean reproductive factors (Rf; final population/ initial population) greater than one (1.3-1.4) for each cultivar in all the population levels. Rf was the highest in cv. Columbian (15.2) for PN with a stylet, followed by cv. Aragorn (12.0) and cv. Cooper (10.8), however, Rf for PN without a stylet was low ranging from 0.9 to 1.1 for three cultivars across the populations. Moreover, proportion of the PN adults with a stylet (15-33%) in the final populations was significantly greater for each cultivar at all the population levels than in the initial populations (< 3%). Morphological and molecular examinations were performed to identify the species of pin nematodes. Morphological measurements of 32 adult females (n = 8/population) included body length (range = 300.2 to 395.5 μ m, mean = 350.1 μ m), stylet length (24.8 to 31.3, 27.6), anterior end to vulva (253.2 to 336.6, 297.2), body diameter (14.3 to 23.4, 17.9), tail length (20.1 to 28.2, 24.7), a (16.9 to 23.5, 19.7), b (3.1 to 4.5, 3.6), c (13.1 to 15.4, 14.1), and V% (83.4 to 86.3, 84.8). Stylet length, body length, V%, individuals lacking a stylet, J-4 and rare males suggested the species to be *Paratylenchus nanus*. DNA was extracted from single nematodes (n = 3) from one field. The 28S D2/D3 region was amplified and sequenced. The sequence was 100% identical to one population of *P. nanus* (KF242199) and 99% identical to three populations of *P. nanus* (KF242198, KF242201, KF242200), confirming the species as *P. nanus*. This research showed that pin nematodes could parasitize these pea cultivars with significant populations of stylet-bearing, feeding adults. The same experiment will be repeated to confirm the research findings.

EFFICACY AND MODE OF ACTION OF *BACILLUS FIRMUS* AS A BIONEMATICIDE FOR THE NORTHERN ROOT-KNOT NEMATODE, *MELOIDOGYNE HAPLA*, AND DAGGER NEMATODE, *XIPHINEMA AMERICANUM*. Valencia, L. and J.B. Kotcon. Division of Plant and Soil Sciences, West Virginia University, Morgantown, WV, 20506.

Root-knot nematodes (RKN) and dagger nematodes (DN) are serious pests of agricultural crops, including peach trees of West Virginia. The endospore-forming bacterium, *Bacillus firmus* (BF), is marketed as a bionematicide, though its mode of action and efficacy in controlling certain nematodes indigenous to WV is unclear. The purpose of this study was to determine efficacy and mode of action of BF as a bionematicide against the northern RKN, *Meloidogyne hapla* and DN, *Xiphinema americanum*. Direct toxicity was determined by exposing RKNs and DN to various concentrations of BF over a 72-hour period. Exposure to a 10⁷ CFU/ml concentration of BF caused a 15% decrease in living RKNs and an 11% decrease in living DN by 72 hours. No effect was observed with lower concentrations. *In vitro* attraction assays were performed to determine if the presence of BF affects nematode migration and infection rates. Filter paper discs were treated with BF and sterile soil extract (SSE) and were placed at either end of a slide covered with Pluronic gel. Approximately 150 RKNs or 30-50 DN

were placed in the center of each slide and the number of nematodes migrating to each side was counted at 1, 2, 4, and 24 hours post inoculation. A similar attraction assay was performed with tomato seedlings in place of filter paper. Filter paper assays showed that 93% of motile RKNs were observed on the SSE portion of the slides compared to 7% on the BF side by hour 24. Tomato seedling assays showed 88% of motile RKNs were observed on the SSE portion of slides compared to 12% on the BF side. DN results were contrary to RKN results. Filter paper assays showed 59% of motile DNs on the BF portion of slides compared to 41% on the SSE side, while tomato seedling assays showed no significant difference between treatments at hour 24. *In vitro* infection assays using RKNs showed an average of 3 RKNs successfully penetrated BF-treated roots compared to 20 in SSE roots. In-sand attraction assays comparing RKN infection of BF and SSE-treated tomato seedlings showed no significant difference between treatments. Nematode mortality observed after exposure to BF suggests that BF produces secondary metabolites that are directly toxic to RKNs and DNs, though these metabolites have limited potency. Behavior of RKNs in the presence of BF suggests the involvement of a chemorepellent, while the behavior of DNs suggests the involvement of a chemoattractant. The results of this study indicate that the mode of action of BF is linked to the production of chemorepellent compounds, though these chemotactic factors are species specific. BF is a promising biocontrol option for the management of RKNs but may not demonstrate the same measure of control against DNs.

EVALUATION OF TEMPERATURE ON ROOT-KNOT NEMATODE INFECTION OF RESISTANT TOMATO CULTIVARS. **Vau, S.J. and D.W. Dickson.** Department of Entomology and Nematology, PO. Box 110620, University of Florida, Gainesville, FL 32611-0620. Corresponding author: svaupor@ufl.edu

Tomato (*Solanum esculentum* L.) grown in warmer climates is susceptible to crop injury by *Meloidogyne* spp. The *Mi-1* gene, first introgressed into cultivated tomato in 1940, is reported to provide an effective source of resistance to *M. arenaria*, *M. incognita* and *M. javanica*. High soil temperatures, however are reported to reduce the gene's effectiveness but there remain considerable inconsistencies in literature regarding soil temperature effects. Our objective was to evaluate different temperature regimes, constant of 28, 32 °C; and diurnal temperatures of 26 °C (12 H) and 32 °C (12 H), on development of *M. arenaria* and *M. javanica*. Two hundred second-stage juveniles (J2) were inoculated on susceptible and resistant tomato cultivars, Agriset and Amelia, respectively; penetration was stopped by washing roots and placed in new sand at the end of 3 days; and three plants were harvest and stained every 2 days until the appearance of females with eggs. At constant temperature of 28 °C, J2 and late J2 of *M. arenaria* (total of 132 and 1, respectively) and *M. javanica* (total of 118 and 25, respectively) were found on Amelia; whereas at a constant temperature of 32 °C all developmental stages from J2 to females with eggs were found in roots of Amelia and Agriset. This suggests that the resistant gene becomes nonfunctional at a constant temperature of 32 °C but remains functional at 28 °C. At 32 C there was a delay of 2 days in the development of egg laying females of *M. arenaria* in Amelia (21 days) compared with that of Agriset (19 days). Under diurnal temperature regime, the resistant gene remained functional. There was a total of 290 and 312 J2 of *M. arenaria* and *M. javanica*, respectively found in Amelia. There was one late stage J2 of *M. javanica* found. In Agriset all developmental stages of *M. arenaria* and *M. javanica* were detected.

NEMATICIDAL POTENTIAL OF *ADENOPHYLLUM AURANTIUM* (MEXICAN ENDEMIC PLANT) FOR CONTROL OF "FALSE ROOT-KNOT NEMATODE" *NACOBBUS ABERRANS* IN TOMATO (*SOLANUM LYCOPERSICUM*). **Velasco-Azorsa, R.¹, I. Cid del Prado-Vera¹, C.B. Hernández².** ¹Colegio de Postgraduados, Mexico Texcoco Mexico state GA 56230. ²Universidad Tecnológica de la Mixteca, Acatlima Oaxaca, Mexico GA. 69000.

In Mexico, the false root-knot nematode, *Nacobbus aberrans*, is distributed in 11 states of the country, where it damages economically important crops such as tomato, chilli and beans. Control of this nematode is mainly with chemical treatments, but there is interest in other tactics (e.g., biofumigation, natural products with nematode toxicity, etc.) to reduce the potential for adverse health and environmental effects of synthetic nematicides. Here we determined the *in vitro* nematicidal activity of compounds isolated from *Adenophyllum aurantium* (Asteraceae), a Mexican plant endemic in the Oaxaca State coastal region. We measured the mobility inhibition (%i) of second-stage juvenile (J2) *N. aberrans* exposed to the methanol extract from roots and aerial parts. The experiment tested extract concentrations of 1000, 100 and 10 ppm, with observations every 12 h up to 72 h and data were subjected to three-way ANOVA and Tukey's HSD. The aerial tissue extract was significantly more bioactive after at 24 h ($P < 0.05$): 94.4 ± 2.2 %i at 1000 ppm and 81.2 ± 4.7 %i at 100 ppm. Juveniles did not recover from the inhibitory effects. The aerial tissue extracts were fractionated with acetone (AcO) and methanol (MeOH). The root extracts were fractionated with ethyl acetate (AcOEt). MeOH was the most potent fraction ($P < 0.05$) causing 100% inhibition without recovery at 50 ppm after 24 h. Column chromatography revealed (1) stigmaterol, (2) beta-sitosterol (AcO partition) and (3) alpha-terthienyl (AcEtO partition), of which compounds 1 (94.4 %i at 36 h) and 3 (91 %i at 60 h) were the most bioactive. Ongoing separation studies of the more active MeOH fraction is expected to yield additional nematicidal compounds according to ¹³C nuclear magnetic resonance spectra. Germination of tomato seeds was inhibited by bioactive concentrations which will require adjustment of application timing. Additional chemical separation of extracts and experiments with tomatoes in pots are ongoing.

EFFECTO DE CUATRO BIONEMATÓXICOS EN EL MANEJO DE *MELOIDOGYNE SPP.* EN *CAPSICUM ANNUUM L.* (EFFECT OF FOUR BIONEMATOTOXIC IN THE MANAGEMENT OF *MELOIDOGYNE SPP.* IN *CAPSICUM ANNUUM L.*) **Paredes, V.¹ and C. Cedano¹.** ¹Facultad de Ciencias Agropecuarias, Universidad Nacional de Trujillo- Perú.

El objetivo del presente trabajo fue determinar el bionematóxico más efectivo en el control de *Meloidogyne spp.* y el momento más oportuno de su aplicación en *Capsicum annuum L.* var. California Wonder desarrollado en macetas con sustrato estéril (mezcla de arena lavada y humus de lombriz en la proporción 2:1) mas 1000 huevos de *Meloidogyne spp.* Los bionematóxicos evaluados fueron Ditera, Nemaquill, Hunter, Nemator y Vidate, aplicados según la dosis comercial en drench (alrededor del cuello de planta) a los 7 y 15 días del trasplante. Además dos tratamientos testigo uno plantas sanas y el otro plantas sanas mas *Meloidogyne spp.* Los tratamientos fueron distribuidos en forma randomizada en un arreglo factorial con 5 repeticiones. El efecto determinado sobre el número de juveniles por 100 cc. de suelo, número de huevos y juveniles por 5 gr de raíces, índice de nodulación, longitud y peso fresco de raíz, peso fresco y seco del follaje y altura de planta. El bionematóxico más efectivo fue Nemathor 20 L en dosis de 1.5 l.cil⁻¹ y el momento más oportuno para la aplicación a los 7 días después del trasplante. Hasta los 66 días de instalado el experimento Nemathor (7 d.d.t.) y Ditera (15 d.d.t.) redujeron la población de *Meloidogyne spp.* a nivel de suelo en 93.1% y 83.4% respectivamente y a nivel radicular en 73.6% y 55%. Los mayores promedios de peso fresco y peso seco aéreo los obtuvo Ditera (15 d.d.t.); mientras que en los parámetros altura de planta, peso fresco y longitud de raíz, no se encontró diferencias significativas entre los tratamientos.

CYTOLOGICAL CHANGES OF EASTER LILY (*LILIUM LONGIFLORUM*) UPON ROOT LESION NEMATODE (*PRATYLENCHUS PENETRANS*) INFECTION. **Vieira, P.^{1,2}, J. Mowery³, J. Kilcrease³, J.D. Eisenback¹, and K. Kamo².** ¹Virginia Tech, Dept. of Plant Pathology, Physiology, and Weed Science, Blacksburg, Virginia, ²USDA ARS, Floral and Nursery Plants Research Unit, BARC, ³USDA ARS, Electron and Confocal Microscopy Unit, BARC.

Lilium longiflorum cv. Nellie White, commonly known as Easter lily, is an important floral crop with an annual wholesale value of over \$20 million in the U.S. The root lesion nematode (RLN), *Pratylenchus penetrans*, is a major pest of lily due to the significant root damage it causes. In this study we investigated the cytological aspects of this plant-nematode interaction using bright and transmission electron microscopy. Phenotypic reactions of roots inoculated with *Pratylenchus penetrans* were evaluated under *in vitro* conditions from 0 to 60 days after nematode infection. Symptom development progressed from initial randomly distributed discrete necrotic areas to advanced necrosis along entire roots of each inoculated plant. The induction and severity of symptoms could be correlated with the number of nematodes (all developmental stages) found parasitizing roots. A major feature characterizing this susceptible-host response to nematode infection was the formation of necrosis, browning, and tissue death involving both root epidermis and cortical cells. Breaking down of consecutive cell walls resulted in loss of cell turgor pressure, lack of cytoplasm integrity, followed by cell death along the route of intracellular nematode migration. Endodermal cells collapsed, forming a physical barrier, and consequently blocking the progression of RLN to parenchymal root tissues. This study presents the first detailed pattern of *P. penetrans* infection of Easter lily, a very important flower crop.

DATA MINING OF THE ROOT LESION NEMATODE (*PRATYLENCHUS PENETRANS*) TRANSCRIPTOME FOR IDENTIFICATION OF CANDIDATE EFFECTOR GENES. **Vieira, P.^{1,2}, T. Maier³, I.A. Zasada⁴, T. Baum³, K. Kamo², and J.D. Eisenback¹.** ¹Virginia Tech, Dept. of Plant Pathology, Physiology, and Weed Science, Blacksburg, Virginia, ²USDA ARS, Floral and Nursery Plants Research Unit, BARC, ³Department of Plant Pathology, Iowa State University, Ames, Iowa, ⁴USDA ARS, Horticultural Crops Research Laboratory, Corvallis, Oregon.

Worldwide crop losses due to plant-parasitic nematodes have been estimated at \$118 billion annually, with *Pratylenchus spp.*, commonly known as the root-lesion nematode (RLN), ranking third in terms of economic losses. Currently, the most common strategies used for RLN control are genetic resistance, nematicide application, and rotation with non-host crops. Host resistance to *Pratylenchus spp.* is very limited, as only a few *loci* have been linked to resistance/tolerance to some RLN species. Application of new technologies to control RLN is needed, since most of the chemicals currently used present negative effects to the environment and increase production costs. Using a comparative transcriptomics approach and a nematode secreted proteins identification pipeline, we identified numerous candidate genes which may be important for the mediation of the interaction of *Pratylenchus penetrans* with its host. The candidate nematode genes represent a range of putative biological functions, such as genes encoding cell wall-degrading enzymes, proteases, putative suppression of the host defenses, and pioneer genes specific to this group of nematodes. In order to validate our *in silico* analyses, a subset of genes were analyzed by semi-quantitative RT-PCR analyses and *in situ* hybridization. The expression patterns of the different candidate genes confirmed their expression *in planta*. Our results revealed the identification of several genes whose expression was restricted to the esophageal glands of *P. penetrans*. This analysis sheds light on putative effector genes of *P. penetrans*, and will aid in the identification of potential gene targets for selection and use to design effective control strategies against root lesion nematodes.

DEVELOPING EFFECTIVE MANAGEMENT STRATEGIES AGAINST PLANT-PARASITIC NEMATODES USING OIL RADISH IN HAWAII. **Waisen, P., K.-H. Wang, Z. Cheng, and B.S. Sipes.** Department of Plant and Environmental Protection Sciences, University of Hawaii at Manoa, Honolulu, HI 96822.

Oil radish (*Raphanus sativus*) is a potential cover crop that has many soil health enhancement properties including nutrient scavenging, soil tilth improvement, fast growth and weed suppressive characteristics. This research focuses on exploring the potential of using oil radish to suppress plant-parasitic nematodes (PPNs) in an agroecosystem in Hawaii. Approaches to develop PPN management using oil radish include: 1) identifying oil radish cultivars susceptible to PPNs commonly found in Hawaii; 2) discovering cultivars with high biofumigation effects when soil incorporated; 3) determining the best cover crop termination time to avoid nematode reproduction; and 4) identifying the best cover crop termination techniques to achieve effective PPN management. This presentation mainly emphasizes on the first three approaches. An oil radish cultivar screening trial was conducted to identify cultivars with higher susceptibility to root-knot nematode, *Meloidogyne javanica*. Eight oil radish cultivars either preferred by Hawaiian growers ('Alpine', 'Discovery', 'Miyashige', 'Oshin', 'April Cross', and 'Summer Cross'), or less expensive seed sold for cover crop ('Sodbuster' and 'Tillage Radish') were planted in pots containing sterile media and inoculated with 2,700 *M. javanica* second stage juveniles per pot. 'Orange Pixie' tomato (*Solanum lycopersicum*) was included as a susceptible control. Although not as susceptible as the tomato, 'Sodbuster', 'Miyashige' and 'Discovery' supported the highest root galling and eggs production one month after nematode inoculation. However, 'Tillage Radish', 'Sodbuster', 'April Cross' and 'Summer Cross' had no *M. javanica* detectable in the soil. Another pot trial was conducted in the greenhouse using field soil infested with *Meloidogyne* spp. and reniform nematode (*Rotylenchulus reniformis*). Soil was amended with leaf tissues of the eight cultivars tested above at 1% (w/w). Tomato 'Orange Pixie' seedlings were planted as a bioassay crop. Unamended soil was included as the control. The experiment was terminated one month after tomato planting. All oil radish cultivars tested significantly reduced populations of nematodes ($P < 0.05$) compared to the unamended control. 'Sodbuster' amendment had the lowest root-knot nematode populations in the soil, whereas no difference in reniform nematode populations was detected among all cultivars. Thus, 'Sodbuster' was a good trap crop for root-knot nematode and an ideal biofumigating crop against root-knot and reniform nematodes. 'Sodbuster' seeds were planted in 40 m² plot at 22 kg seeds/ha in a root-knot and reniform nematode infested field. 'Sodbuster' roots were sampled and stained for nematode penetration weekly over an 8-week period. Soil was collected simultaneously from the rhizosphere and bare ground area to examine nematode populations in the soil. Data loggers were buried at 10-cm deep to monitor soil temperature to determine heat units required to reach egg laying females for both nematodes. It is anticipated that this will help to determine the best 'Sodbuster' termination time to avoid nematode reproduction, thus achieving effective trap cropping and biofumigating effects.

SUPPRESSION OF SOYBEAN BAG6 INDUCED PROGRAMMED CELL DEATH BY SOYBEAN CYST NEMATODE *HETERODERA GLYCINES* EFFECTORS. **Wang, J.¹, G. Yeckel¹, P.K. Kandoth¹, L. Wasala¹, R.S. Hussey², E.L. Davis³, T.J. Baum⁴ and M.G. Mitchum¹.** ¹Division of Plant Sciences and Bond Life Sciences Center, University of Missouri, Columbia, MO 65211, USA; ²Department of Plant Pathology, University of Georgia, Athens, GA; ³Department of Plant Pathology, North Carolina State University, Raleigh, NC; ⁴Department of Plant Pathology and Microbiology, Iowa State University, Ames, IA.

Plant pathogens deliver diverse effector proteins into host cells to suppress plant defense and promote infection. While numerous effectors that suppress plant immunity have been identified from bacteria, fungi, and oomycete pathogens, relatively little is known for nematode effectors. During an incompatible interaction with soybean, SCN triggers a hypersensitive response (HR)-like programmed cell death (PCD) at the feeding sites within soybean roots. The mechanism by which virulent SCN populations overcome this incompatibility is unknown. *GmBAG6-1*, a soybean *BAG6* (Bcl-2 associated athanogene 6) gene, is highly upregulated in SCN feeding sites undergoing a HR-PCD, but is suppressed by a virulent SCN population. Like its *Arabidopsis* homolog *AtBAG6*, *GmBAG6-1* induces PCD in yeast and also in soybean. Thus, we utilized a yeast viability assay to screen several dozen SCN stylet-secreted effector candidates for their ability to specifically suppress *GmBAG6-1* induced cell death. We identified several effectors that strongly suppressed cell death mediated by *GmBAG6-1*. Two effectors identified as suppressors showed direct interaction with *GmBAG6-1* suggesting that one mechanism of cell death suppression may occur through direct interaction with this host protein as part of their strategy to overcome soybean resistance. Identification of a set of plant defense suppressors in SCN will facilitate ongoing investigations of the underlying functions of nematode effector proteins in nematode pathogenesis.

RELATIONSHIPS BETWEEN COVER CROP PLANT AVAILABLE NITROGEN MINERALIZATION RATE AND NEMATODE SOIL HEALTH INDICATORS. **Wang, K.-H., S. Ching, J. Marquez, S. Mishra, P. Waisen, and Z. Cheng.** Dept. Plant and Environmental Protection Sciences, University of Hawaii at Manoa.

Cover crop calculators have been developed by Oregon State University based on Vigil and Kissel's (1991) equation for the temperate maritime and semi-arid region to estimate plant available nitrogen (PAN) mineralized from cover crop residues. These calculators are not completely applicable to tropical climates due to differences in PAN mineralization rates (PAN%)

affected by climate, soil type, cover crop species, biomass, plant age, %N in cover crop tissue, farming practice and microbial activity in soil. Hawaii has a wide range of soil orders, microclimates, and farming practices, thus a statewide program was carried out to estimate PAN% from various key agriculture production areas. Cover crops with different %N were incubated in field soil at 25% soil moisture and 25 °C at 1% (w/w) amendment rate based on dry weight content over a 28-day period. Regression lines between PAN% (y) and %N of cover crop residue (x) generated from Poamoho on Oahu ($y = 14.9x + 25.6$, $R^2 = 0.76$, $P < 0.001$), Waimea on the Big Island ($y = 37 + 5.9x$, $R^2 = 0.50$, $P < 0.001$), Ho'olehua on Molokai ($y = -15.6x^2 + 123.5x - 145$, $R^2 = 0.55$, $P < 0.0001$), and Kula on Maui ($y = -6.2x^2 + 49x - 32.1$, $R^2 = 0.50$, $P = 0.0042$) were projecting higher PAN% than that estimated by Vigil and Kissel's equation. Tabulating these equations into a calculator format to calculate actual PAN (actual PAN = cover crop biomass based on dry weight \times PAN%) allows farmers to estimate the amount of fertilizer they could reduce. However, a comparison of PAN% of the same cover crops incubated in Poamoho soil in winter tilled (WT), winter no-till (WNT), and summer no-till (SNT) conditions revealed differences in PAN% from the same soil. Multivariate analysis between PAN% of individual cover crops and the nematode community indices (abundance of bacterivores, fungivores, herbivores, omnivores and carnivores, enrichment index, structure index, channel index, genus richness and diversity) of the unamended soil revealed significant correlation between these parameters. This paper will present the magnitude of difference in PAN% among soils with different nematode community trajectories.

CURRENT STATUS OF MICHIGAN SOYBEAN CYST NEMATODE TYPES. Warner, F.¹, A. Tenney¹, and G. Bird².
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Heterodera glycines (soybean cyst nematode) is a key limiting factor in U.S. soybean production. It was detected in more than 50% of Michigan's soybean acreage in 2011-2012. Shortly after *H. glycines* was discovered in North Carolina in 1954, it became apparent that populations varied in their abilities to feed and reproduce on resistant varieties of *Glycines max*, and a Race Test was developed to differentiate among populations. More recently, HG and SCN Type Tests were developed to assess the phenotypes of SCN populations. In 2014-2015, Michigan State University, Diagnostic Services performed 59 SCN Type tests. Sixty-one percent of the populations were classified as Type 2; whereas, 32%, 2%, and 5% were classified as Types 1.2, 1 and 0, respectively. None were classified as Type 4. This indicates that varieties derived from PI 88788 are no longer resistant to 94% of the populations evaluated. Based on their Female Indices, 47%, 26% and 4% of the populations were slightly, moderately or highly aggressive in regards to their abilities to reproduce on PI 88788. These data were used to develop a conceptual crop loss assessment model for the impact of *H. glycines* on Michigan soybean production under current nematode management practices.

DISCOVERY AND CHARACTERIZATION OF WOLBACHIA IN PRATYLENCHUS PENETRANS. Wasala, S.¹, A.M.V. Brown¹, A.B. Peetz², D.K. Howe¹, I.A. Zasada², and D.R. Denver¹.
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Wolbachia is an endosymbiotic bacterium known to infect numerous filarial nematode and arthropod species. Prior to this work, *Wolbachia* has been observed in only one plant-parasitic nematode, *Radopholus similis*. *Wolbachia* is an exciting target for environmentally-friendly strategies for the control of parasites and disease vectors that harbor it. In arthropods, *Wolbachia* can affect host biology, ecology, and evolution by manipulating host reproduction. By contrast, in filarial nematodes *Wolbachia* are obligate mutualists providing essential nutrients to their hosts. The extent to which *Wolbachia* infect different plant-parasitic nematode species remains unknown, as does their impact on host biology. We initially discovered the presence of *Wolbachia* in another plant parasitic nematode, *Pratylenchus penetrans*, using an Illumina-based genome skimming approach. To confirm and further investigate the presence of *Wolbachia* within *P. penetrans*, we developed *Wolbachia*-specific Fluorescence In-Situ Hybridization (FISH) probes and PCR detection assays. These tools were then used to analyze for the presence of *Wolbachia* in ten *P. penetrans* populations from commercial raspberry production fields in Washington, USA. Prevalence of *Wolbachia* in male and female nematodes and their genetic differences within populations were also characterized. Our study confirmed the presence of *Wolbachia* within 4 of the 10 *P. penetrans* populations investigated. FISH imaging revealed that *Wolbachia* is localized within the head region, pharynx, gut, and ovaries of the nematode. For the infected populations, the prevalence of *Wolbachia* was not 100% fixed, as is observed in the filarial nematodes. Rather, the *Wolbachia* prevalence within populations varied between 10% to 50% indicating that this endosymbiont is not an obligate mutualist of *P. penetrans*. While *Wolbachia* was observed in both male and female nematodes, there was a clear female-biased sex ratio in *Wolbachia* infected *P. penetrans* populations, indicating *Wolbachia* might act as a reproductive manipulator inducing male killing or feminization of biological males. In one *P. penetrans* population, we observed many divergent *Wolbachia* 16S rRNA sequence types between nematodes, and also within a single nematode. This suggests that there might be substantial *Wolbachia* genetic variation in this single nematode population. By contrast, another *P. penetrans* population had *Wolbachia* 16S rRNA sequences that were 100% identical in all nematodes. These findings build on the first report of *Wolbachia* in *P. penetrans* and reveal new basic biological and genetic insights into the nature of the *Wolbachia* – *P. penetrans* symbiosis.

OBSERVATIONS ON THE EFFICACY OF BIOLOGICAL AND NATURAL PRODUCTS FOR MANAGING PLANT PARASITIC NEMATODES. **Westerdahl, B.B.** Department of Entomology and Nematology, University of California, Davis CA.

The availability of several biological and natural products for managing plant parasitic nematodes has increased in recent years. Traditional management practices have focused on increasing yields by reducing nematode populations. Field trials with a number of the newer products on annuals, perennials and ornamentals have demonstrated that in some cases they are effective in improving plant growth and yield either without reducing, or at times even increasing nematode populations. Possible reasons for these results include plant growth stimulating properties, and healthier root systems being able to support greater nematode populations. These findings also present challenges with grower acceptance of the newer products.

DEVELOPMENT OF WALNUT ROOTSTOCKS RESISTANT AND TOLERANT TO NEMATODES. **Westphal, A.¹, T.R. Buzo¹, M. McKenry¹, Z.T.Z. Maung¹, F. Westphal¹, G.T. Browne², C. Leslie³ and D. Kluepfel².** ¹Dept. Nematology, UC Riverside, Parlier, CA 93648; ²USDA-ARS Crops Pathology and Genetics Research Unit, Dept. Plant Pathology, UC Davis, Davis, CA 95616; ³Dept. Plant Sciences, UC Davis, Davis 95616.

Walnut orchards can be economically productive for 35 to 50 years. This longevity necessitates proper decision-making and management prior to planting. For years, soil fumigation has been instrumental in reducing attack by bacteria, fungi, nematodes and water molds. The loss of methyl bromide and restrictions of 1,3-D containing materials increases reliance on host plant resistance to mitigate negative impacts by soil-borne pathogens. The aim of this consortium of scientists from the USDA-ARS, UC Davis, and UC Riverside is to develop superior rootstocks that can withstand pathogen attack. For this purpose, different crosses of *Juglans californica*, *J. cathayensis*, *J. hindsii*, *J. major*, *J. microcarpa*, *J. nigra* and *J. regia* are prepared. Clonal offspring of these crosses are then tested for response to crown gall, Phytophthora root rot, root-knot and root lesion nematodes. In nematode tests, seedlings or clones are planted into fields, and inoculated with *Pratylenchus vulnus* and *Meloidogyne incognita*. These plants are monitored for vigor and nematode reproduction for several years. While nematode reproduction on such plants after one year is often minimal in a large percentage of tested entries, nematode reproductive values differ more in the second year. The second monitoring year was shown to be fully indicative of beneficial plant characteristics that then can be confirmed in the third year of sampling. These breeding efforts have identified a number of promising candidates that are tested further. The over-arching goal of the program is producing superior rootstocks requiring fewer agronomic inputs. Ultimately, this will increase tree longevity and reduce the need for post-plant remedial actions.

REPRODUCTION OF *MESOCRICONEMA XENOPLAX* AND *PRATYLENCHUS VULNUS* ON PISTACHIO. **Westphal, A., T.R. Buzo, Z.T.Z. Maung, and M. McKenry.** ¹ Dept. Nematology, UC Riverside, Parlier, CA 93648.

Pistachio is one of the expanding nut crops in California. In previous surveys in the 1980s, rare infestations of orchards with plant-parasitic nematodes and limited risk for damage were attested. Changes in the genetic material incorporated in rootstock development and the rapid increases of acreage warrant further exploration of the nematode host status of currently used rootstocks. The likelihood of pistachio to be planted into nematode-infested ground increases following row crops or a nut crop orchard. In this project, the susceptibility to two different populations of *Pratylenchus vulnus* (standard or aggressive) on the popular 'UCB1' pistachio rootstock clone was compared to various Prunus rootstocks. UCB1 was somewhat less susceptible compared to Prunus rootstocks but known aggressive population *P. vulnus* was also more aggressive on UCB1. When *P. vulnus* reproduction on UCB1 was compared to that under the highly resistant rootstock 'Krymsk1', an apparent interaction with soil texture of the growth substrate and nematode co-infestation with *Mesocriconema xenoplax* was observed. Two greenhouse experiments with UCB1 each in a factorial design with the three factors (1) soil texture: sand or sandy loam, (2) inoculation with *P. vulnus*: none, standard or aggressive population, and (3) inoculation with *M. xenoplax*: absent or present were conducted. In these studies, sand allowed for higher reproduction of *M. xenoplax* than sandy loam. Nematode numbers of the aggressive population of *P. vulnus* per gram of root were higher than for the standard population. On average, plants of the two experiments co-inoculated with *M. xenoplax* supported higher *P. vulnus* than the non-inoculated equivalents. The number of *P. vulnus* was independent of the soil texture and suggested to be a nematode-to-nematode interaction. There is the potential risk for build-up of *P. vulnus* and *M. xenoplax* on UCB1, and care needs to be taken when pistachio on specific clones of the UCB1 rootstock is planted into soils with multiple nematode species present.

EFFECTOR GENE DISCOVERY IN THE RENIFORM NEMATODE THROUGH GENOME SEQUENCING AND RNA-SEQ OF LIFE-STAGES. **Wubben, M.J.¹, K.C. Showmaker², W.S. Sanders², S. Eves-van den Akker³, M.A. Arick II², Z.V. Magbanua², and D.G. Peterson².** ¹USDA-ARS, 810 Highway 12 East, Mississippi State, MS 39762, ²Institute for Genomics, Biocomputing, and Biotechnology, Mississippi State University, Mississippi State, MS 39762, ³Division of Plant Sciences, College of Life Sciences, University of Dundee, Dundee, DD1 5EH, United Kingdom.

The reniform nematode (RN; *Rotylenchulus reniformis*) occupies a unique evolutionary niche among plant-parasitic nematodes (PPNs), having a feeding strategy between the migratory and sedentary endoparasitic lifestyles. As a sedentary semi-endoparasite, RN expresses a suite of genes that initiate and maintain a permanent feeding site in the host root. Initial

efforts to identify these parasitism genes, now known as 'effector' genes, in RN were based solely on homology with well-characterized effectors from other plant parasites. Recently, we completed the assembly and annotation of the reniform nematode genome which was facilitated by high-throughput sequencing of RNAs from all RN life-stages. BLASTP analysis identified a variety of RN effector homologs including chorismate mutase, ubiquitin extension proteins, and venom allergen-like proteins as well as multiple PPN 'pioneer' effectors. In previous work we had identified three RN genes homologous to CLAVATA3/ESR peptides (CLE); however, manual curation of the RN genome identified 17 more CLE-like genes. A similar approach identified 13 members of the C-terminally Encoded Peptide (CEP) hormone mimics which have been implicated in modulating host plant nitrate uptake. In addition to sequencing, a replicated differential expression (DE) analysis was performed between the non-infective second-stage juvenile (J2) and parasitic sedentary female (SF) life-stage. DE analysis showed that approximately one-fourth of all transcripts were differentially expressed between J2 and SF. Furthermore, within this group of transcripts were 64 that showed homology to previously identified nematode effectors. Next, a bioinformatic pipeline was created to identify putative novel RN effectors based on the following characteristics: lack of homology to known proteins, predicted extracellular secretion, lack of transmembrane domains, and up-regulated expression in the SF life-stage. In total, 384 RN proteins met all of the criteria; however, when only two criteria were met the total number of proteins increased approximately 10-fold, indicating a potentially massive collection of proteins involved in RN parasitism.

SUSCEPTIBILITY OF GRAIN SORGHUM CULTIVARS TO *MELOIDOGYNE INCOGNITA* ISOLATES FROM LOUISIANA. **Xavier-Mis, D.M.¹, F.M.C. Godoy¹, C. Overstreet¹, and E.C. McGawley¹.** ¹LSU AgCenter, Dept. Plant Pathology and Crop Physiology, Baton Rouge, LA 70803.

There are conflicting reports relating to host susceptibility of grain sorghum (*Sorghum bicolor*) to *Meloidogyne incognita*. Grain sorghum can potentially be utilized as a rotation crop with cotton and soybeans. Greenhouse studies were conducted with grain sorghum cultivars grown in Louisiana to evaluate their susceptibility to *Meloidogyne incognita*. A preliminary experiment was done with 29 sorghum cultivars tested against one Louisiana isolate of *M. incognita*. There was a range of susceptibility to *M. incognita* among the sorghum cultivars tested, with final populations of nematode ranging from 10,500 to 500 juveniles/500 cm³ soil 60 days after inoculation. Three grain sorghum cultivars Dekalb DKS53-67, REV 9924, and REV 9782, considered very susceptible, moderately susceptible and very resistant, respectively, according to the first experiment were selected for further testing. In a second greenhouse experiment, the three sorghum cultivars previously selected were tested with 10 isolates of *M. incognita* from different Louisiana Parishes. Among all the grain sorghum and nematode isolate combinations tested, reproduction by *M. incognita* isolate 19 (RK19) on DKS53-67 cultivar was the highest of the group with over 24,000 juveniles/500 cc of soil. Nematode reproduction of RK19 isolate on REV 9924 and REV 9782 cultivars was 2.6 and 3.7 fold lower than on DKS53-67 cultivar, respectively. The lowest nematode reproduction was observed on RK6 on REV 9782 cultivar, which was about 10 times lower than the same isolate reproduction on DKS53-67 cultivar. The same pattern of nematode reproduction observed for juveniles/500 cc of soil was also found in number of eggs/g of roots for the nematode isolates and sorghum cultivars combinations. Although there were significant variations in reproduction among the nematode isolates with very susceptible and moderately susceptible cultivars, there were no differences in reproduction among isolates on the resistant cultivar REV 9782. In Louisiana, many of the fields where sorghum could be grown have both *Rotylenchulus reniformis* and *M. incognita* nematodes. Therefore, a cultivar needs to be used in a rotation that would be effective in reducing both nematodes rather than only one species.

LIFE HISTORY EVOLUTION OF AN ANTARCTIC NEMATODE: ELEMENTAL STOICHIOMETRY AND THE GROWTH RATE HYPOTHESIS. **Xue, X.¹, B.N. Adhikari², A. Perkes,^{1,4} M. Martin^{1,5}, D.H. Wall³ and B.J. Adams¹.** ¹Department of Biology and Evolutionary Ecology Laboratories, Brigham Young University, Provo, UT, USA. ²USDA-ARS, Tucson, Arizona, USA ³Department of Biology and Natural Resource Ecology Laboratory, Colorado State University, Fort Collins, CO, USA. ⁴Department of Biology, University of Pennsylvania, Philadelphia, PA, USA. ⁵Arizona College of Osteopathic Medicine, Midwestern University, Glendale, AZ, USA.

Elemental stoichiometry is a powerful integrator of biological and geochemical evolution, provides a useful framework for understanding sources and controls of nutrient availability, and has been widely applied in the study of different ecosystems, including the Antarctic Dry Valleys. Prior *in situ* research on natural populations of the Antarctic soil nematode *Plectus murrayi* revealed a link between cellular phosphorus (P) and organismal development as postulated by the growth rate hypothesis (GRH). This hypothesis infers that high biomass P-content reflects an increased allocation to P-rich ribosomal RNA, and is needed to meet the protein synthesis demands of development. In accordance with the GRH, we hypothesize that in a P-limited environment, animals will grow more slowly but achieve a larger body size at maturity. We also predict that in a P-deficient environment we will find lower cellular RNA concentrations and that natural selection will reduce the number of copies of RNA genes in the genome, and subsequently lower rates of overall gene expression. To test the GRH in *P. murrayi* under laboratory conditions, we manipulated the amount of available P to see if we could replicate in the laboratory the pattern previously identified in Dry Valley field conditions, and to see if we could identify some of the specific mechanisms

connecting elemental constraints and ontogeny. Because even under the best conditions field and laboratory-reared populations of *P. murrayi* are relatively slow growing, we replicated our experiments with the more rapidly growing nematode, *Caenorhabditis elegans*. Our experimental evolution results for *C. elegans* are consistent with resource availability and the GRH. We found that the number of copies of the 18S ribosomal DNA tandem array in *C. elegans* cultured in a P-limited environment is 13 times less than populations reared in a P-enriched environment. Under similar conditions, *P. murrayi* also evolved a decrease in rDNA gene copy number, although not as dramatic. Additionally, the adult body size of both *C. elegans* and *P. murrayi* reared in excess P was significantly smaller than those reared in P-limited conditions. Our findings underscore the important relationship between the evolution of life history traits and genome organization, as well as the role of elemental stoichiometry in shaping the organization of trophic interactions and, ultimately, ecosystem structure and functioning.

FIRST DETECTION OF *PRATYLENCHUS SCRIBNERI* ON POTATO AND *P. NEGLECTUS* ON WHEAT IN NORTH DAKOTA. **Yan, G.**¹, **A. Plaisance**¹, **D. Huang**¹, and **Z.A. Handoo**². ¹North Dakota State University, Department of Plant Pathology, Fargo, ND 58108, ²USDA-ARS, Nematology Laboratory, Beltsville, MD 20705.

Root-lesion nematodes (*Pratylenchus* spp.) are the most common nematode pests of field crops. In 2014 and 2015, 54 soil samples were collected from a potato field in Sargent County, ND to investigate the occurrence of root-lesion nematodes. Nematodes were extracted from each soil sample, and 48 of the samples contained root-lesion nematodes with population densities ranging from 125 to 1,900/kg of soil. The root-lesion nematodes were identified as *Pratylenchus scribneri* using morphological and molecular methods. One soil sample with 1,540 root-lesion nematodes/kg soil was used to inoculate potato cultivar All Blue. After ten weeks of growth at controlled greenhouse conditions, the population of root-lesion nematodes was found to have increased substantially (average = 9,163/kg soil + 48/g roots), indicating that this root-lesion nematode reproduced well on this potato cultivar. Root-lesion nematodes extracted from both soil and potato roots in the greenhouse were tested and confirmed as *P. scribneri*. Similarly, five soil samples were collected in 2015 from a wheat field in Walsh County, ND and were found to have root-lesion nematodes from 125 to 1,044/kg soil. Morphological and molecular examinations identified the root-lesion nematodes as *P. neglectus*. One soil sample with 500 root-lesion nematodes/kg soil was used to inoculate hard red spring wheat cultivars Glenn and Faller. After 10 weeks of growth, wheat roots were harvested and washed, and the root-lesion nematodes were able to be recovered from the root tissues. Averages of 24 and 20 root-lesion nematodes per g were found in the roots of Glenn and Faller, respectively. Nematodes were isolated from both soil and wheat roots, and were confirmed as *P. neglectus*. In comparison, the sequence of 28S D2/D3 expansion domain of *P. neglectus* had 79% similarity with that of *P. scribneri*, demonstrating that these two species have a big variation in this genomic region. The key morphometric differences between these two species from North Dakota were the percentage of position of vulva (V%) from anterior end to the total body length (*P. neglectus*: range = 81.0 to 85.0%, mean = 82.8%; *P. scribneri*: 75.5 to 78.7, 77.2) and tail length (*P. neglectus*: 16.0 to 22.0 μ m, 18.8 μ m; *P. scribneri*: 25.0 to 28.0, 25.6). Other morphological measurements between adult females of these two species overlapped to some extent, including body length, stylet, body width, anterior end to basal bulb, total body length divided by maximum body width (a), total body length divided by pharyngeal length (b), and total body length divided by tail length (c). Several *Pratylenchus* species including *P. scribneri* are detrimental to potato. *P. neglectus* has been reported as a damaging pathogen that affects wheat production in the Pacific Northwest. This represents the first occurrence of *P. scribneri* and *P. neglectus* in North Dakota.

RESISTANCE AND TOLERANCE OF SOME ONION CULTIVARS TO *DITYLENCHUS DIPSACI* (KUHN, 1937). **Yavuzaslanoglu, E.** Karamanoglu Mehmetbey University, Technical Sciences Vocational School, Plant and Animal Production Department, Karaman, Turkey.

Onion is a cross pollinated plant grown all over the world especially in temperate regions. Turkey onion production is in 6th place after China, India, USA, Iran and Russia in the world. Total annual onion production is 1.904.846 tonnes with 298,584 hg/ha yield in 2013. One of the biotic constraints of onion is stem and bulb nematode; *Ditylenchus dipsaci* (Kühn, 1937) distributed in common onion growing areas in Turkey. Yield loss due to the *D. dipsaci* was recorded up to 41.5% on commonly grown onion cultivars. The current study was conducted to investigate the resistance and tolerance reaction of some commercial cultivars. Totally 24 commercially grown onion cultivar were screened for resistance and tolerance reactions to *D. dipsaci* under growth room conditions at 20 °C, 70% humidity, and 16 hours day light. Two hundred nematodes were inoculated onto 5 week-old seedlings in 10 μ l 1% CMC solution with 4 replications for each cultivar. Plants were grown for 5 weeks after nematode inoculation. Multiplication rate (MR) of Valenciana cultivar was 0,46 and partially resistant. Multiplication rate of other cultivars were between 3-92,5 being susceptible. Infected seedlings showed increase in plant and leaf diameter, reduction in plant height and softening of plant tissue. There was no significant difference among cultivars for tolerance reactions. Plant diameter was significantly positively correlated to leaf diameter, plant fresh weight and total number of leaves per plant. Total number of *D. dipsaci* and MR were significantly positively correlated to plant diameter.

MOLECULAR CHARACTERISATION FOR ADVANCING ROOT-KNOT NEMATODE SYSTEMATICS. **Ye¹, W.**
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Root-knot nematodes (*Meloidogyne* spp.) are the most economically damaging genus of plant-parasitic nematodes on horticultural and field crops. They are distributed worldwide and are obligate parasites of the roots of thousands of plant species, including monocotyledonous and dicotyledonous, herbaceous and woody plants. The genus includes more than 100 species with some species having several races. From 2006 to 2016, a total of 786 root-knot nematode populations were collected from North Carolina field crops, ornamental plants and turfgrasses. Root systems showing galling symptoms were dissected under the microscope and females were obtained for DNA analysis. Since some of these samples were submitted as soil only, the second-stage juveniles or males were used instead. Some infested soil samples were reared in tomato in greenhouse to get the females. Molecular characterisation was performed by DNA sequencing on the ribosomal DNA 18S, ITS and 28S D2/D3, intergeneric spacer, RNA polymerase II large subunit, mitochondrial DNA cytochromas gene subunit II and histone gene H3. Eight species were identified, including *M. incognita* (prevalence 75.1%), *M. enterolobii* (6.7%), *M. hapla* (5.3%), *M. marylandi* (4.1%), *M. arenaria* (3.7%), *M. graminis* (2.2%), *M. javanica* (1.8%), and *M. naasi* (0.4%). *M. enterolobii* and *M. marylandi* were reported from North Carolina for the first time. Species-specific primers were developed to identify some root-knot nematode through simplex or duplex PCR. Molecular diagnosis using PCR by species-specific primers provides a rapid and cheap species identification approach for root-knot nematodes.

THE GENUS *DELADENUS* (TYLENCHIDA: NEOTYLENCHIDAE). **Yu, Q.¹, L.J. Haavik², W. Ye³, and J. Gu⁴.**
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In recent years, there have been enhanced interests in studying *Deladenus*, and using them as potential biocontrol agents for controlling insect pests. Recent studies suggest that all strains are not created equal: North American strains of *D. siricidicola* didn't sterilize their host females as the Kamona strain does; and those seemingly species-specific interrelationships among the *Deladenus*, *Sirex*, and *Amylostereum* are not so specific after all. With new species discovered, and described; new knowledge suggests that taxonomic revisions of the genus are necessary. Since the ground-breaking discovery of those species of *Deladenus* with infective stage by Bedding in the late 60s, at least 4 new species have been described. In addition, molecular data have been added. This paper reviews the biodiversity, distribution, and taxonomy of the species of the genus.

EVALUATION OF SOIL TEMPERATURE ON SUSCEPTIBILITY OF CV. TIFGUARD PEANUT TO *MELOIDOGYNE ARENARIA*. **Yuan, W.¹, C.C. Holbrook², Y. Chu³, P. Ozias-Akins³, and D.W. Dickson¹.** ¹Entomology and Nematology Dept., University of Florida, Gainesville, FL 32611. ²Crop Genetics and Breeding, USDA-ARS, Tifton, GA 31793. ³Dept. of Horticulture, University of Georgia, Tifton, GA 31793. Corresponding author: ywm@ufl.edu

Tifguard was released in 2008 as a highly resistant peanut cultivar to *Meloidogyne arenaria* race 1. During the summer of 2012 root-knot nematodes were found damaging Tifguard in 14 field sites in Levy and Marion counties, Florida. Our objective was to determine the role of soil temperature on the susceptibility of Tifguard to root-knot nematode infection. Environmental controlled chambers were used to evaluate the effect of temperature on nematode infection rate and development. All Tifguard seeds were processed for determination of resistant gene marker before the experiment. Seedlings of resistant and susceptible cvs. Tifguard and Georgia-06G were inoculated with 1,000 freshly-hatched second-stage juveniles (J2) of *M. arenaria*. Tomato cv. Agriset 334 with the same treatment was included to assure inoculum viability. The peanut and tomato seedlings were divided into three groups based on different incubation temperatures of 28, 31, and 34 °C. Every 5 days after inoculation the root systems from three plants of each cultivar and tomato from each temperature were cleared and stained with acid fuchsin. The number of nematodes in roots and their developmental stages were determined. Overall, the temperature significantly affected the nematode infection and development in the three hosts ($P \leq 0.05$). Rate of development of nematodes in tomato was much greater than that in peanut at all three temperatures. At the first sampling date the number of J2 that penetrated roots at 31 and 34 °C was less in Tifguard than in tomato and Georgia-06G peanut ($P \leq 0.05$). J2 that entered Tifguard roots increased significantly as temperature increased ($P \leq 0.05$). Egg-laying females were generally observed 15 to 20 days after inoculation in Georgia 06 and tomato roots at all three temperatures. Although J2 were able to penetrate Tifguard roots they did not undergo further development at 28 or 31°C, however at 34°C a few J3-J4, females, egg laying females, and males of *M. arenaria* were observed. The egg masses on Tifguard were small and contained only a few eggs relative to numbers found in egg masses removed from Georgia-06G ($P \leq 0.05$). In summary, high soil temperatures could lessen the effectiveness of the nematode resistance gene in Tifguard.

CURRENT STATE OF *GLOBODERA* INFESTATIONS IN THE U.S. **Zasada, I.A.¹, X. Wang², and L.M. Dandurand³.**
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Potato cyst nematodes, *Globodera pallida* and *G. rostochiensis*, are globally important nematode parasites of potato and both are considered quarantine pests in most countries. In the United States, *G. rostochiensis* was detected in New York in the 1940s and *G. pallida* was more recently detected in Idaho in 2006. In addition to these detections, a new species of *Globodera*, *G. ellingtonae*, was described from populations collected in Oregon and Idaho. Combined, the presence of these *Globodera* pose a threat to the \$3 billion U.S. potato industry. The regulatory stories of *G. pallida* and *G. rostochiensis* in the United States provide a framework in which to evaluate the pros and cons of regulatory and management/eradication practices used to contain these economically important plant-parasitic nematodes. The use of quarantine and delimitation methods, plant resistance, soil fumigation, and alternatives to fumigation in the United States against potato cyst nematodes will be discussed.

NEMATODOS FITOPARASITOS ASOCIADOS AL CULTIVO DE QUINUA (*CHENOPODIUM QUINOA* WILLD.) EN LA REGION PUNO. **Zavalla-Tapia, N.¹, M.C. Zheyla¹, L.M. Israel¹, B.P. Rosario¹, A.G. Marilia¹, C.J. Shadam¹, G.A. Sthewart¹, and F.Ch. Yeni¹.** ¹Universidad Nacional del Altiplano, Puno, PE.

Actualmente el Perú es el principal productor y exportador de quinua a nivel mundial; su cultivo en la zonas alto andinas tienen por objeto satisfacer necesidades básicas y asegurar la nutrición de la población constituyendo un cultivo estratégico para contribuir a la seguridad y soberanía alimentaria; sin embargo la producción de este grano se ve afectada por plagas y enfermedades que provocan daños en las hojas, el tallo, raíces y la panoja; el presente trabajo tiene por objetivo Identificar la presencia de nematodos fitoparásitos en las zonas cultivadas de quinua en la región Puno – Perú; para lo cual se analizaron 293 muestras colectadas en la campaña agrícola 2015-2016 procedentes de 12 distritos de mayor producción de quinua en la región Puno. Las muestras fueron procesadas por el método de fluctuación centrífuga en solución de sacarosa. La identificación de géneros se realizó mediante lecturas con un estereoscopio y la ayuda de patrones fijados en lámina. Los Géneros identificados fueron los siguientes; *Globodera* spp., *Mesocriconema* spp., *Helicotylenchus* spp., *Xiphinema* spp., *Nacobus* spp., *Pratylenchus* spp., *Meloidogyne* spp., *Discocriconema* spp., *Dorylaimus* spp., *Tylenchus* spp., *Rotylenchus* spp., *Hoplolaemus* spp., *Hemicycliophora* spp., *Mononchus* spp., y *Rhaphiditis* spp. El género de mayor prevalencia fue *Globodera* spp. en los distritos de Cabana y Chucuito (73,8 y 72,56% respectivamente) seguido del género *Mesocriconema* spp. en los distritos de Ilave (57.43%) y Azángaro (42,93%) estando distribuido en todas las zonas evaluadas; de la misma manera los resultados muestran la presencia de nematodos de vida libre en todas las muestras analizadas.