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ABSTRACTS

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CHARACTERIZATION OF ANTAGONISTIC RHIZOBACTERIA TO CONTROL *MELOIDOGYNE JAVANICA* ON TOMATO. **Abadir, Seham K., A. E. Tawfik, and F. W. Raid.** Plant Pathology Institute, Agriculture Research Center, Giza, Egypt.

The impact of different antagonistic rhizobacteria on reduction of disease caused by Meloidogyne javanica was studied and effective strains were selected. *Bacillus subtilis, Pseudomonas fluorescens* and *Serratia marcescens* were evaluated against the root-knot nematode, *M. javanica* infecting tomato plants. All antagonistic bacteria were used by adding 100 ml broth culture adjusted to 10^8 cfu/ml for each treatment. Both *P. fluorescens* and S. *marcescens* isolates were most effective in nematicidal activity against hatched juveniles of *M. javanica* in vitro, thus the mortality levels of second stage juveniles increased being 100% compared with the control being 5.5% after 24 hours. Under greenhouse conditions, the number of nematode galls and egg masses were decreased when the antagonistic bacteria were introduced prior to nematodes and *S. marcescens* was found to be the most effective bacteria. It is noticeable that the reduction of gall formation, and number of egg masses on tomato roots reflected on the increase percentage of fresh weight of foliage and roots. Furthermore, it can be concluded that plant parasitic nematode *M. javanica*, was subjected to a wide range of antibiotic producing antagonists *P. fluorescens* or alkaline volatile products produced by *S. marcenens*.

NEMATICIDAL ACTIVITY OF SOIL MICROORGANISMS' METABOLITES. **Abdel-Rahman, F. H., R. H. Nsaif, and S. I. Massoud.** Department of Biology, Texas Southern University, Houston, TX 77004.

Twenty five species of soil microorganisms (bacteria, and fungi) were isolated and identified from agricultural soil. Biolog microstation and data base systems were used for identifying all bacterial isolates. Light and Scanning Electron Microscopes were used in fungal identification. SEM/X-Ray Detector was used to determine the elemental composition for each isolated fungus. Some of the tested bacterial species were *Pseudomonas aeruginosa, P. fragi, Burkholderia gladioli, B. capacia, Bacillus cereus/thuringiensis, B. thermoglucosidasius, B. amyloliquefaciens, Serratia odorifera, S. marcescens, Alcaligenes latus*, and *Agrobacterium tumefaciens*. Some of the fungal isolates were *Aspergillus niger, A. flavus, A. clavatus, Fusarium solani, Helminthosporium sp., Plasmodium sp., Rhizopus sp.,* and *Monilia* sp. A comprehensive bioassay was carried out to test all isolated microbial metabolites for their nematicidal activity. Second-stage larvae of the root-knot nematode were incubated in microbial filtrates/metabolites in the water-screen bioassay, and observed using the stereomicroscope. Several bacterial and fungal species caused high mortality of the second-stage larvae in the wet-screen bioassay. In another bioassay the second-stage larvae of root-knot nematode were incubated in microbial filtrates for 48 hours and then introduced to healthy tomato seedlings, nematode incubated in water served as control. All plants were kept in the green house and/or in the growth chamber. After two months all root- systems were removed and the root-gall index were determined. It has been found that several bacterial and fungal species reduced the ability of second-stage larvae of root-knot nematode from penetrating, infecting, and/or developing on the tomato roots as it was indicated from the root-gall index. Metabolites/filtrates of the fungus *Aspergillus niger;* and the bacteria, *Bacillus cereus, B. amyloliquefaciens, Serratia odorifera*, and *Burkholderia cepacia*, proved to possess higher nematicidal activity.

VIRULENCE OF ENTOMOPATHOGENIC NEMATODES TO THE CITRUS ROOT WEEVIL: A COMPARISON OF INDIGENOUS AND COMMERCIALLY AVAILABLE NEMATODES. **Adolphson, D.,[1] K. B. Nguyen,[2] and B. J. Adams.**[1] [1]Microbiology and Molecular Biology Department, Brigham Young University, Provo, UT 84602; [2]Entomology and Nematology Department, University of Florida, Gainesville, FL 32611.

The citrus root weevil, *Diaprepes abbreviatus*, is a major pest of citrus crops in Florida and the Caribbean. Currently, entomopathogenic nematodes are being used as biological control agents against the weevil. However, it has been shown that indigenous nematodes that are locally adapted can provide improved control and persistence in Florida citrus agroecosystems. Our objective was to conduct a laboratory comparison of several entomopathogenic nematode strains that are native to Florida and are distributed near major citrus growing areas. Each strain was tested against larval *D. abbreviatus* with larval mortality determined 15 days after inoculation. Each treatment consisted of 10 experimental units (ten con-

tainers of one larva each per nematode strain) and was replicated three times. Significance among different nematode strains was determined using arcsine transformation and analysis of variance, as well as a t-test.

INTRASPECIFIC VARIABILITY OF *ROTYLENCHULUS RENIFORMIS* FROM COTTON-GROWING REGIONS IN THE UNITED STATES. **Agudelo, Paula,[1] R. T. Robbins,[1] J. McD. Stewart,[2] and A. L. Szalanski.[3]** [1]Departments of Plant Pathology, [2]Crop, Soils and Environmental Sciences, and [3]Entomology, University of Arkansas, Fayetteville, AR72701.

Reniform nematode (*Rotylenchulus reniformis*) is a major pest in cotton production in the southeastern United States. The objective of this study was to examine the variation of reniform nematode populations from cotton-growing locations in the United States where it is prevalent. Multivariate analysis of variance and discriminant analysis were used to determine the variability of morphology in males and immature females. Reproduction indices of populations were measured on selected soybean and cotton hosts in the greenhouse. High variability in morphometrics and reproduction was observed within all the populations, and several differences were found among populations. DNA sequences of the nuclear ribosomal first internal transcribed spacer region (ITS1) were compared among US populations and to sequences of populations from Brazil, Colombia, Honduras and Japan. No polymorphic nucleotide sites were observed among the amphimictic populations. Only a parthenogenic population from Japan was distinct. The phenotypic polymorphism of the species in the United States could impact the effectiveness of management strategies based on host plant resistance. The overlapping of phenotypic data and the lack of molecular genetic distinction suggests a recent geographical expansion from a relatively small base of origin.

DEVELOPMENTAL EXPRESSION PROFILES FOR THE MJ-CM GENE FAMILY. **Akraiko, Tatsiana, and K. N. Lambert.** Department of Crop Sciences, University of Illinois, Urbana, IL 61801.

Root-knot nematodes (*Meloidogyne* spp.) are obligate plant parasites that cause severe problems in many annual and perennial crops worldwide. One gene that may play several roles in nematode parasitism is chorismate mutase (CM). CM is thought to manipulate the plant's shikimate pathway and to be involved in suppression of plant defenses and the alteration of plant development. Three novel genes belonging to the *Meloidogyne javanica* chorismate mutase gene family have been cloned and studied. Those are *Mjcm-0, Mjcm-2 and Mjcm-3*. The length of proteins encoded by these 3 genes ranges from 140 aa (*Mjcm-0*) to 266 aa (*Mjcm-3*). Partial cDNA sequence of *Mjcm-4* gene was also obtained and used for establishment of transcript expression profile of this gene. Real-time quantitative RT-PCR method was used to generate transcript expression profiles of four members of *Meloidogyne javanica* chorismate mutase gene family over seven developmental timepoints in the nematode life cycle: egg, infective second stage juveniles (Inf-J2), 2-day after inoculation (ai), 4-day ai, 7-day ai, 14-day ai, and adult. In this study, differences in the nematode mRNA levels for each gene were observed over the *M. javanica* lifecycle. Comparison of transcript profiles showed that *Mj-cm-0* and *Mj-cm-1* are highly expressed and *Mj-cm-2* and *Mjcm-4* are expressed at a low level. A transcript copy number of *Mjcm-3* has not been quantified by real-time quantitative RT-PCR, but analysis of regular RT-PCR results suggests that *Mjcm-3* is most likely a low abundance gene. Currently we are developing developmental expression profiles of gene transcripts of interest in virulent *M. javanica*. We hypothesize that variation in expression of some members of CM gene family in virulent versus avirulent nematodes might play a role in nematode virulence.

CROSS PROTECTION INVOLVING VIRULENT AND AVIRULENT *MELOIDOGYNE* SPP. ATTACKING A GRAPE ROOTSTOCK. **Anwar, S. A, and M. V. McKenry**, Department of Nematology, University of California, Riverside, CA 92521.

Root tips of Harmony grape (*Vitis champinii* × 1613C) develop a hypersensitive response when penetrated by juveniles from common *Meloidogyne incognita* populations. *Meloidogyne incognita* and *M. arenaria* already familiar with Harmony rootstock are able to overcome this resistance mechanism. The interaction of an avirulent population of *M. incognita* and a virulent population of *M. arenaria* was assessed on a split-root system of Harmony grape. Five thousand eggs of an avirulent *M. incognita* were applied to one half of the split-root. At, 0, 7, 13, 20, and 27 days after inoculation (DAI) the other half received 5,000 eggs of virulent *M. arenaria*. In a second experiment, influence of the population density of an avirulent population on the interaction was tested by exposing half the split-root-system with 0, 500, 1,500, 5,000, and 10,000 eggs 15 days before applying a challenge-inoculation of 5,000 eggs of *M. arenaria*. Control treatments for each experiment involved half the root system being non-inoculated. Each treatment was replicated five times. Reproduction potential of each nematode population was assessed using Pf/Pi where Pf = final number of eggs per root system 90 DAI. Cross protection was evident in both experiments. The avirulent *M. incognita* suppressed reproduction of virulent *M. arenaria* by 23%, 72%, 74% and 74% in the 7, 13, 20, and 27 DAI compared to their respective inoculated controls. Cross protection increased by 9%, 26%, 76% and 81% relative to the non-inoculated control at 500, 1,500, 5,000 and 10,000 eggs per plant when compared to the non-inoculated control. These results indicate that feeding attempts by an avirulent population can induce plant defense reactions that virulent populations normally circumvent.

INVESTIGATION OF NEMATOPHAGOUS FUNGI IN SOIL AND ON ROOTS USING REAL- TIME PCR. **Atkins, S. D.,[1] B. Peteira,[2] I. M. Clark,[1] P. R. Hirsch,[1] B. R. Kerry.[1]** [1]Nematode Interactions Unit, Rothamsted Research, Harpenden, UK; [2]Centro Nacional de Sanidad Agropecuria, Havana, Cuba

Real time PCR (QPCR) using SYBR green or Taqman probes is a powerful tool in detecting, monitoring and quantifying fungi directly from soil, and when used in combination with other detection methods such as baiting and serial plate dilutions on selective media provides valuable data on the abundance, activity and efficacy of released biological control agents. Taqman probes and primer sets have been developed for the nematophagous fungi *Paecilomyces lilacinus, Plectosphaerella cucumerina, Pochonia chlamyosporia* var. *chlamydosporia* and *Pochonia chlamydosporia* var. *catenulata*. The Taqman assays were highly sensitive, being able to detect accurately population sizes as low as five spores g^{-1} soil. Probes and primer sets have also been produced for specific isolates of *Pochinia chlamydosporia* var. he specie specific Taqman assays have been used to investigate the abundance and activity of the different species in two sites in the UK where soil has been shown to be naturally suppressive to potato cyst nematodes, and to track the release of *Pochonia chlamydosporia* var. *catenulata,* in field trials to control root knot nematodes in Cuba. Interactions in the rhizosphere and root knot nematode egg masses was also investigated between different varieties and isolates of the fungus to try and detect competition between fungal isolates in the niches occupied. The use of real time PCR has enabled the complex interactions between different and closely related fungal taxa to be investigated, which has not been possible with earlier techniques.

IDENTIFICATION OF LANCE NEMATODE WITH PCR-RFLP OF ITS1. **Bae, C. H.,[1] R. T. Robbins,[1] and A. L. Szalanski.[2]** [1]Departments of Plant Pathology and [2]Entomology, University of Arkansas, Fayetteville, AR 72701.

Lance nematode *Hoplolaimus* spp. is an important nematode of various crops in the Southeastern United States. The Columbia lance nematode, *H. columbus* is an economically important nematode of cotton, corn, and soybean in Alabama, Georgia, North Carolina, and South Carolina. In this study, specimens of *H. columbus* from Louisiana, South Carolina, and Georgia, *H. galeatus* from Florida, Louisiana, and South Carolina, and *H. magnistylus* from Arkansas were obtained. These species are closely related taxonomically. Overlap of some important characters makes them difficult to identify from morphological and morphometrical data, especially when only a few individuals are detected. To improve diagnostic accuracy, a simple and convenient identification method is needed. DNA sequencing and PCR-RFLP analysis were used to characterize the first internally transcribed spacer region (ITS1) of these three *Hoplolaimus* species. Interspecies and intraspecies ITS1 size variation was not detected. Differences found in ITS1 sequences of the three lance species were used to infer phylogenetic relationships among them. PCR-RFLP of ITS1 was used to detect several restriction enzyme site differences between populations and species.

FIELD ESTIMATES OF COFFEE YIELD LOSSES AND DAMAGE THRESHOLD BY *MELOIDOGYNE EXIGUA*. **Barbosa, Dimmy, H. S. G. Henrique, D. Vieira, Ricardo M. Souza, Alexandre P. Viana, and Carina P. Silva**. Universidade Estadual do Norte Fluminense, Av. Alberto Lamego, 2000, Campos dos Goytacazes (RJ), Brazil.

In a first approach to assess yield losses and damage threshold by *M. exigua*, 125 coffee plantations in the Northwest region of the State of Rio de Janeiro, Brazil, were categorized in two ages and three technological levels (I to III), according to the growers' use of fertilization, soil amendments, and proper control of weeds, pests and diseases. For each group, data on the productivity of the last five years and on nematode population (measured as second-stage juveniles [J2] in the soil) in the last two years, were analysed through univariate and multivariate statistics. The results indicated that *M. exigua* was not a major cause of the low productivity in poorly or just fairly managed plantations (technological levels II and III). On the contrary, the best managed plantations (technological level I) had no tolerance to *M. exigua* populations as low as 10 to 15 J2/100 cc. of soil, with populations of 40 J2/100 cc. of soil causing as much as 45% yield loss.

NEMATODE-CROP DAMAGE-INTRODUCTION. **Barker, K. R.** Plant Pathology Department, North Carolina State University, Raleigh, NC 27695.

One of the earliest studies on nematode-crop damage involved J. Kühn's 1880's assessments of multiple tactics (rotation, antagonistic plants, and soil fumigation [carbon disulfide]) for control of *Heterodera schachtii* on sugarbeet. Other early landmark research on crop damage and mechanisms of pathogenesis included E. A. Goeldi's findings that *Meloidogyne exigua* predisposed coffee to *Rhizoctonia solani*, and G. F. Atkinson's similar findings on *M. incognita*'s interaction with *Fusarium* wilt on cotton. Later work showed that *M. incognita* and other species circumvent host resistance to other pathogens, whereas some nemas, including *Heterodera glycines*, suppressed the activities of symbionts (such as *Bradyrhizobium japonicum* and *Glomus* spp). Nema-crop damage may be restricted by certain rhizobacteria by the latter's inducing host resistance to nemas. Key methods for assessing nematode populations were developed by N. A. Cobb (1918), while G. Thorne in the 1920's explored the utility of such data for managing *H. schachtii* on sugarbeet. The discovery of effective nematicides and nema-resistant plant genotypes in the 1940'–50's catalyzed intense studies on nema-crop damage. Resulting developments included nema-population models (F. G. W. Jones and J. W. Seinhorst) and crop-damage functions/nema-host models (F. G. W. Jones, J. W. Seinhorst, M. Oostenbrink). Early nema-population and/or nema-host crop

simulators were developed by F. G. W. Jones (*Globodera rostochiensis*), and H. Ferris (*Meloidogyne* spp.). Recent applications of GPS and GIS technologies for assessing/managing site-specific crop damage show promise, but require further automation for increased utility. Major challenges remain in the characterization and management of nema-crop damage/yield losses. Among critical needs are efficient/automated nema identifications and quantifications, more precise/predictable characterizations of nema-host-soil/general environment interactions, and greater understanding of related soil biology. Still, interfacing the rapidly evolving new technologies with given field histories and comprehensive cropping-IPM systems offers exciting opportunities.

NEMATODE CONTROL VS. PLANT PROTECTION. **Becker, J. O.** Department of Nematology, University of California, Riverside, CA 92521.

Chemical strategies against plant parasitic nematodes target three spatial zones: the bulk soil, the areas surrounding the crop plant surface or its inside tissues. Soil fumigation destroys nematode pests in crop-free soil with high application rates. The second strategy became feasible only after the development of non-fumigant nematicides that allowed at- or after-planting treatments. Band application, side-dressing or bed chemigation dramatically reduced the nematicide rates as the goal shifted from nematode elimination to crop protection. Compounds with systemic movement provide limited curative action. Nematicide seed treatments were briefly tested but never gained widespread acceptance. This might have been primarily because of the highly toxic nature of the tested compounds and the limited protection period compared to longer-lasting soil-applied nematicides. Today, many of the carbamate and organophosphate nematicides that were evaluated as seed treatments are banned or are targeted by legislative regulations. On the other hand, hybrid seed has become increasingly expensive. High quality seed is already routinely treated with fungicides and/or insecticides. The addition of a compatible, efficacious nematicide would improve the protection package and provide the best possible start for young seedlings under disease and pest pressure. This strategy is not likely to be always as effective as broadcast soil application but in many instances it may be an economically and ecologically sensible key ingredient of a holistic IPM approach.

SELECTION OF HGCM-1 ALLELES OF *HETERODERA GLYCINES* ON RESISTANT SOYBEANS. **Bekal, S.,[1] N. Atibalentja,[1] T. L. Niblack,[1] G. R. Noel,[1,2] C. Smyth,[1] and K. N. Lambert.[1]** [1]Department of Crop Sciences, University of Illinois at Urbana-Champaign, Urbana, IL 61801; [2]USDA/ARS, Urbana, IL 61801.

The soybean cyst nematode (SCN), *Heterodera glycines*, is the most destructive pathogen of soybean in the Unites States. Diversity in the parasitic ability of the nematode allows SCN to frequently grow on resistant soybean. Hgcm-1 is a SCN chorismate mutase, which is thought to function as a candidate virulence gene. Hgcm-1A and Hgcm-1B are two members of the Hgcm-1 gene family that have been isolated from avirulent and virulent inbred SCN. In this study, we conducted controlled crosses between two inbred SCN lines to generate a F2 SCN segregating population for Hgcm-1A and B. This population was then selected on resistant (PI88788, PI90763, Hartwig) and susceptible (Lee 74) soybeans and genotyped using a real-time PCR (QPCR) assay. The QPCR analysis showed that SCN growing on PPI88788 were predominately Hgcm-1A type as a result of a statistically significant drop of Hgcm-1B allele in the population. No significant difference was observed in SCN population grown on other resistant soybeans compared to the susceptible plant. Hg-cm-1A and 1B genes are highly repetitive in the SCN genome. The observation that they segregate approximately in a 1:2:1 ratio indicates Hg-cm-1 consists of a block of tandemly arrayed genes that segregate as a single locus.

PREY PREFERENTIAL BEHAVIOR IN A DIPLOGASTERID PREDATOR MONONCHOIDES N.SP. **Bilgrami, A. L., and R. Gaugler.** Department of Entomology, Rutgers University, New Brunswick, NJ 08901.

Nine species of plant-parasitic nematodes were subjected to predation to determine predator's preference for prey. Prey species was subject to predation singly and in all possible paired combinations separately to obtain absolute preference (AP%), relative acceptance (RA%) and rejection (RR%) of prey, strike rate of predators (SR%), prey resistance (PR%) and prey susceptibility (PS%) to predation. The second stage juveniles of *Meloidogyne incognita* and *Heterodera mothi* were most (SR = 92–94%; AP = 80–85%; PS = 91–93%; PR = 7–9%) and *Helicotylenchus indicus* the least preferred (SR = 60%; AP = 35%; PS = 50%; PR = 50%) prey species. The predator preferred *M. incognita* and *H. mothi* (RA = 32–36% and RR = 0–4%) over other species in all prey combinations. *Hirschmanniella oryzae* and *Longidorus attenuatus* were preferred moderately over other prey nematodes with RA of 24–28%. When given a choice of prey predators rejected *Helicotylenchus indicus* (RR = 36% and RA = 0%). No individual of *Hoplolaimus indicus* or *H. mangiferae* was killed (RR = 100%). Present study showed that predators preferred second-stage juveniles of *M. incognita* and *M. mothi*. The reasons for prey preference may be correlated to search ability and strike rate of the predator and physical, chemical and behavioral characteristics of prey individuals providing resistance against predation.

THE FIRST 24 HOURS: PRIMARY SIGNALING EVENTS BETWEEN ROOT-KNOT NEMATODE AND ITS HOST. **Bird, D. McK.,[1] R. R. Weerasinghe,[2] N. S. Allen,[2] and D. P. Lohar.[1]** Center for the Biology of Nematode Parasitism[1] and Department of Botany,[2] North Caroline State University, Raleigh, NC 27695.

Root-knot nematodes (RKN: *Meloidogyne* spp.) are significant agricultural pests worldwide, and induce stereotypical giant cells (GC) in the root vasculature of most plants. The discovery that GC express *KNOX* and *PHAN* transcription factors required for meristem maintenance led us to hypothesize that GC are a type of induced meristem, perhaps with similarities to those induced by rhizobia, which also express *PHAN* and *KNOX*. Our finding that the nodule-regulation genes *ccs52* and *ENOD40* also are expressed in young GC strengthened this idea. Additionally, rhizobia and RKN both hyperinfect a *Lotus japonicus har1-1* null. This gene encodes the CLAVATA1 receptor kinase, which plays a central role in maintaining meristem size in *Arabidopsis*. Computational analysis (Olsen and Skriver, 2003) revealed a potential CLAVATA3-like peptide in a cyst nematode EST dataset. CLAVATA3 is the presumed ligand for CLAVATA1, although formal proof that the nematode product interacts with the plant receptor is yet to be obtained. Genetic evidence points to a role for *KNOX* in regulation of hormonal response pathways, and cytokinins have been implicated in the formation of nodules. We used the cytokinin-responsive *Arabidopsis ARR5* promoter driving *GUS* to obtain a spatio-temporal map the cytokinin response in *Lotus*. A cytokinin response was observed in curled/deformed root hairs shortly after interaction with rhizobia and was evident at the earliest development stages of the nodule primordium, with expression declining as the nodule emerged from the root. In contrast, a cytokinin response was not detectible during root penetration and migration by RKN, nor in mature GC. Down-regulation of cytokinin levels *in planta via* transgenic expression of cytokinin oxidase genes produces roots with significantly fewer nodules. However, the number of feeding sites induced by RKN also was reduced, even though established GC do not mount a detectible cytokinin response. One interpretation is that a spike of cytokinin is required at the onset of GC initiation, but not for GC maintenance. Confocal microscopy of *Lotus* composite plants transgenic for GFP-marked actin and microtubules revealed that RKN elicit cytoskeletal responses in root hairs indistinguishable from those induced by rhizobia/Nod factor. RKN also induce root hair deformation and branching. Because they encode primary rhizobial signal receptors, we have begun to dissect the host pathway which responds to the diffusible RKN signal using *Lotus symRK, nrf1* and *nrf5* mutants. We found that NFR1 and NFR5 are required for RKN induced root hair branching, but SYMRK is not. A separate, wavy-root hair phenotype is unaltered in the three receptor mutants tested, suggesting either that RKN produces two signals, or that the RKN signal functions in the plant at two steps. Physical characterization of these signaling molecules will likely provide significant insight into the RKN-plant interaction and may shed new light on rhizobial-plant interactions.

YIELD ADVANTAGES OF *HETERODERA GLYCINES* RESISTANT VARIETIES, SOIL ELECTRICAL CONDUCTIVITY, AND HG-TYPES ASSOCIATED WITH MICHIGAN SOYBEAN PRODUCTION. **Bird, G., N. Miller, F. Warner, and A. Tenney.** Michigan State University, East Lansing, MI 48824.

Equipment with real-time yield and soil electrical conductivity monitoring systems were used to assess advantages of SCN resistant varieties in eight commercial soybean fields in MI. Each site was planted to alternate strips of susceptible and PI 88788-based varieties, and mapped in relation to bean yield, SCN population density and soil electrical conductivity. An additional site was used to monitor HG-Types associated with multi-year planting schemes of three sources of SCN resistance. Yield advantages associated with SCN resistant varieties exhibited extensive within-field variation, with the greatest advantage occurring in areas of high SCN population densities and low soil electrical conductivity. After four years of continuous SCN susceptible, PI 54840, PI 88788, or PI 437654-based varieties; a mixture of susceptible, PI 54840 and PI 88788-based varieties; or a three-year rotation with susceptible, PI 54840 and PI 88788-based varieties, the HG- Type 2.5.7 was present when PI 88788 was used in the system. HG-Type 5.7 was present in the continuous PI 54840 plots. At-harvest population densities of SCN associated with PI 437654 were too low for collection of enough nematodes for a HG-Type Test.

RATES OF MOLECULAR EVOLUTION IN ANTARCTIC AND TEMPERATE NEMATODES. **Bliss, T. J.,[1] B. J. Adams,[1] and U. Gozel.[2]** [1]Department of Microbiology and Molecular Biology, Brigham Young University, Provo, UT 84604; [2]Entomology and Nematology Department, University of Florida, Gainesville, FL 32611.

While it is well established that different species appear to evolve at different rates across evolutionary time, correlations between the rate of evolution and biological or ecological constraints are poorly understood. Nematodes from the Antarctic Dry Valleys have long generation times, but shorter periods of time to complete their life cycle compared to closely related species from temperate regions. Thus, we predict that Antarctic nematodes should evolve more slowly than sister taxa in warmer climates that are able to complete more generations over the same period of time. To test this hypothesis we sequenced ribosomal genes from Antarctic Nematodes and compared their rates of molecular evolution with sister taxa from more northerly distributions. Compared to other nematodes in the phylum Nematoda, *Eudorylaimus antarcticus* appears to evolve significantly slower than its counterparts. However, when the comparison is constrained to account for phylogenetic autocorrelation, evolutionary rate differences disappear. The extent to which these patterns hold up across the phylum may provide an understanding of the role of rates of molecular change in the mode and tempo of nematode evolution.

RESEARCH GAPS AND CHALLENGES IN THE USE OF NEMATODE COMMUNITIES IN ENVIRONMENTAL STUDIES. **Bongers, T.,[1] and H. Ferris.[2]** [1]Laboratory of Nematology, Wageningen University, P.O. Box 8123, 6700 ES, Wageningen, The Netherlands; [2]Department of Nematology, University of California, Davis, CA 95616.

The impact of a stressor or disturbance is usually expressed in changes in dominance within a nematode community. One measure of the impact of disturbance is the Maturity Index (MI). The MI is based on a classification (the cp series) of nematode families with regard to reproductive rate and life history characteristics. As experience is gained with application of the MI, it has become apparent that not all taxa within a family behave similarly in response to stress. Some taxa may have to be excluded from the index or have changes made in their coefficients. Calibration of stress response at the species level will undoubtedly result in refinement of the MI and derived indices. New molecular techniques provide opportunities to unravel phylogenetic relationships and to strengthen the ecological basis for assessment of environmental conditions. The techniques offer alternatives to laborious methods of species determination, allow reliable identification of juvenile stages, and do not limit the number of specimens that can be identified with available resources. However, molecular techniques are not yet sufficiently developed for quantitative measure of all species in the nematode community. High priority research gaps and challenges include the need to develop indices less dependent on the abundance of each taxon so that the use of molecular techniques is facilitated. Moreover, there is need to develop reference systems to allow assessment of the magnitude of a stress, the effects of complex disturbances and for more dependable determination of food preferences at lower trophic levels.

ATTACHMENT AND DEVELOPMENT OF *PASTEURIA PENETRANS* IN *MELOIDOGYNE MAYAGUENSIS*. **Brito, Janete A.,[1] R. Cetintas,[2] J. D. Stanley,[1] J. F. Preston,[3] and D. W. Dickson.[2]** [1]Division of Plant Industry, Gainesville, FL 32614-7100; [2]Department of Entomology and Nematology, University of Florida, Gainesville, FL 32611-0620; [3]Department of Microbiology and Cell Science, University of Florida, Gainesville, FL 32611-0700.

Pasteuria penetran, is an endospore-forming prokaryotic parasite of *Meloidogyne* spp. Two isolates of *P. penetrans* originally collected from *Meloidogyne arenaria* race 1 (P-20), and *Meloidogyne* spp. (P1-UFLA), and an isolate attained from *Pratylenchus scribneri* (B-4) were evaluated for their ability to attach to and develop in three isolates of *M. mayaguensis*. *M. arenaria* race 1, *M. incognita* race 4, and *M. javanica* were used as susceptible hosts. Attachment tests were performed by a centrifugation method using 1-day-old second-stage juveniles (J2) and endospores at a 1:140 ratio. None of the isolates of *Pasteuria* were compatible with *M. mayaguensis*, however attachment, propagation, and maturation were observed in all the nematodes used as controls.

REPRODUCTION OF *MELOIDOGYNE MAYAGUENSIS* FROM FLORIDA ON ROOT-KNOT NEMATODE RESISTANT TOMATO AND PEPPER. **Brito, Janete A.,[1] J. D. Stanley,[1] R. Cetintas,[2] M. Di Vito,[3] J. A. Thies,[4] and D. W. Dickson.[2]** [1]Division of Plant Industry, Gainesville, FL 32614-7100; [2]Department of Entomology and Nematology, University of Florida, Gainesville, FL 32611-0620; [3] Istituto per la Protezione delle Piante, Sezione di Bari, C.N.R., Via Amendola, 165/A, 70126 Bari, Italy; [4]U.S. Vegetable Lab. USDA, ARS, 2875 Savannah Highway, Charleston, SC 29414-5334.

Meloidogyne mayaguensis is a highly virulent pathogen of many crops including tomato and pepper. Isolates of this pathogen collected in other countries have been reported to reproduce on *Mi-1* gene resistant tomato, which provides resistance to the major root-knot nematode species. Studies were conducted to determine the reproduction of an *M. mayaguensis* isolate from Florida on root-knot nematode resistant tomato and pepper. *M. incognita* race 4 was used as a control. Four resistant tomato genotypes BHN543, BHN 585, BHN 586, and Sanibel were compared with the susceptible 'Rutgers' tomato, and four resistant pepper genotypes Charleston Bell, Lines 9913/2, 97.9001, and 97.9008 were compared with the susceptible 'Resistant Giant' pepper. Tomato was tested at 22 and 33 °C in growth chambers, and at 26 ± 1.8 °C in a growth room. *M. mayaguensis* reproduced on all *Mi-1* gene tomato at all temperatures, whereas *M. incognita* reproduced only at 33 °C. Pepper, which was tested at 24 °C, was infected only by *M. mayaguensis*. Galling and egg mass indicies, and the reproduction factor for *M. mayaguensis* were significantly different than those for *M. incognita* race 4 in all resistant tomato and pepper genotypes. *M. mayaguensis* from Florida overcame the *Mi-1* resistance gene in tomato genotypes, and *N* resistance gene on pepper 'Charleston Bell,' and other gene(s) that confers resistance to root-knot nematodes on pepper lines 9913/2, 97.9001, 97.9008.

IDENTIFICATION AND HOST PREFERENCE OF *MELOIDOGYNE MAYAGUENSIS*, AND OTHER ROOT-KNOT NEMATODES FROM FLORIDA, AND THEIR SUSCEPTIBILITY TO *PASTEURIA PENETRANS*. **Brito, Janete A.,[1] J. D. Stanley,[1] R. Cetintas,[2] T. O. Powers,[3] R. N. Inserra,[1] E. J. McAvoy,[4] M. L. Mendes,[2] W. T. Crow,[2] and D. W. Dickson.[2]** [1]Division of Plant Industry, Gainesville, FL 32614; [2]Department of Entomology and Nematology, University of Florida, Gainesville, FL 32611; [3]Department of Plant Pathology, University of Nebraska, Lincoln, NE 68583-0722; [4] Vegetable/Ornamental Horticulture, University of Florida, P.O. Box 68, La Belle, FL 33975.

Root-knot nematode species were identified from 465 soil and root samples primarily by isozyme analysis (EST and

MDH), however morphology and mitochondrial DNA analyses were used when needed. The species identified were *Meloidogyne arenaria, M. graminicola, M. hapla, M. incognita, M. javanica, M. mayaguensis*, and *Meloidogyne* spp. *Meloidogyne mayaguensis* occured sympatrically with *M. arenaria, M. incognita*, and *M. javanica. Pasteuria penetrans* was not found infecting *M. graminicola* or *M. mayaguensis*. In Florida, *M. mayaguensis* has been found in roots of basil (*Ocimum* sp.), bell pepper [*Capsicum annuum* var. *annuum* (Grossum Group)], egg plant (*Solanum melogena*), guava (*Psidium guajava*), and tomato (*Lycopersicon esculentum*). Several ornamental plants found as host included ajuga, (*Ajuga reptans*), angel trumpet (*Brugmansia* 'Sunray'), cape honeysuckle (*Tecomaria capensis*), crimson bottlebrusth (Callistemon citrinus), glorybower (Clerodendrum ugandense), glory bush (*Tibouchina* 'Compacta' and *Tibouchina elegans*), lantana (*Lantana* sp.), and wax myrtle (*Myrica cerifera*). Also, two wild plant species, American black nightshade (*Solanum americanum*) and wild poinsettia (*Poinsettia cyathophora*), were found heavily infected with *M. mayaguensis*. In a differential host test this nematode showed the same reaction as *M. incognita* race 4.

HUMAN DISTURBANCE AND NEMATODE DECLINE IN ANTARCTIC DRY VALLEY SOIL. **Broos, E. J.,[1] D. H. Wall,[1] R. A. Virginia,[2] J. N. Nkem,[1] L. E. Powers,[3] and B. J. Adams.[4]** [1]Natural Resource Ecology Laboratory, Colorado State University, Fort Collins, CO 80523; [2]Environmental Studies Program, Dartmouth College, Hanover NH 03755; [3]Department of Engineering, University of Wisconsin, Madison ,WI 53706; [4]Microbiology and Molecular Biology Department, Brigham Young University, Provo, UT 84602.

The McMurdo Dry Valleys of Antarctica contain the simplest soil communities on earth but may be threatened by increasing human disturbance through tourism, scientific activity and climate change. Most human activity in the dry valleys consists of movement to sites by foot. In this study, over a period of eight years we examined the effect of heavy and light trampling (foot paths) on soil nematode communities. Nematode communities were sampled to 10 cm depth from soil in trampled areas and compared to communities in immediately adjacent undisturbed areas. In the last year of sampling the effect of trampling on nematode communities at varying soil depth (0 to 2.5 cm and 2.5 to 10 cm) was also investigated. Overall, total nematode abundance was lower in trampled areas than undisturbed areas ($P < 0.05$). The effect was most significant in the last year of sampling when nematode abundance was 71% lower in trampled areas than undisturbed areas. Eudorylaimus antarcticus were 74% more abundant in areas with light trampling than heavy trampling ($P < 0.05$). Over the eight year duration of the experiment nematode abundance was constant in the heavily trampled areas but fluctuated in the lightly trampled and undisturbed areas ($P < 0.05$). Differences in nematode communities between trampled and undisturbed areas were observed both in samples taken to 2.5 cm and to 10 cm depth but were more significant in the surface samples. These results indicate Antarctic soil nematode communities are sensitive to human disturbance by trampling and effects increase with duration of disturbance.

THREE-DIMENSIONAL RECONSTRUCTION OF COMPLEX EXTRACELLULAR APPENDAGES IN *ACROBELES COMPLEXUS* (CEPHALOBINAE). **Bumbarger, D.,[1] J. G. Baldwin,[1] and John Crum.[2]** [1]Department of Nematology, University of California, Riverside, CA 92521; [2]NCMIR at University of California, San Diego, CA 92093.

Acrobeles complexus is a microbial feeding nematode common in many soil types and globally distributed. Prominent and highly complex cuticular appendages, termed probolae, surround the mouth opening and are presumably associated with feeding. The 3-dimensional internal morphology of probolae and the interface with hypodermal cells is examined with both electron tomography and reconstruction from serial thin section transmission electron microscopy. High-pressure freezing and freeze substitution were utilized to preserve the integrity of hypodermal membranes and protein laminates within the cuticle. Observations are discussed in the context of cuticular structures in other groups, including the cephalic framework of Tylenchida nematodes. Testable hypotheses are provided for plausible processes of probolae.

FUNCTIONAL ANALYSIS OF POTENTIAL NEMATODE RESISTANCE GENES. **Burgwyn, Beth, Ray Collier, and Christopher G. Taylor.** Donald Danforth Plant Science Center, St. Louis, MO 63132.

Composite plants, comprised of wild type shoots with transgenic roots, are a valuable tool for the functional analysis of genes involved in plant-parasitic nematode interactions. An efficient, non-sterile, non-tissue culture method has been developed that enables researchers to quickly produce composite plants for testing. This method of generating composite plants has been demonstrated on numerous species of dicotyledonous plants. *Agrobacterium rhizogenes* was used to introduce the hairy root T-DNA along with a T-DNA containing an RNA interference (RNAi) construct to examine the role of genes during nematode infestations. Visualization using GFP enabled selection of transgenic roots. Obligate plant-parasitic nematodes, such as soybean cyst nematode or root-knot nematode were cultivated on RNAi expressing composite plants. As an initial test of gene functional analysis, we have silenced known nematode resistance genes in resistant plant varieties to determine if silencing can confer a susceptible phenotype. Feeding site formation and reproduction of root-knot nematode on *Mi* silenced tomato demonstrates that this method is useful in determining if a gene confers nematode resistance. This method is currently being applied to other genes that are presumed to play a role in nematode resistance based on their identification through mapping of QTL.

FLUORESCENT ANTIBODY LABELING OF ADHERENS JUNCTIONS AS A TOOL FOR INVESTIGATING PHYLOGENETICALLY SIGNIFICANT MORPHOLOGICAL CHARACTERS. **Burr, A. H. Jay,[1] and James G. Baldwin.[2]** [1]Department of Biological Sciences, Simon Fraser University, Burnaby, BC, Canada V5A 1S6; [2]Department of Nematology, University of California, Riverside, CA 92521.

We are investigating stoma structures as well as other cellular characters to be evaluated in combination with sequence data to improve phylogenetic analysis of higher taxa. This approach has been useful for understanding the relationships of Rhabditina, Bunonematidae, Diplogasterida and Cephaloboidea, however obtaining the detailed structural information required reconstruction from serial TEMs. Such work is so costly and lengthy that it inhibits investigation of the many other species needed to understand uncertain relationships among the higher nematode taxa. Here we describe a promising method for fluorescent antibody visualization of cell junctions along the stoma. We will demonstrate the resolution of the method by comparing our fluorescent images with published TEMs of the head of C. elegans and other species. After being fixed and permeabilized, worms are stained by binding MH27 to adherens junctions and then binding Cy3-labeled anti-IgG to MH27. MH27 cross-reacts with widely divergent nematode species. Many species can be processed simultaneously over two to three days and then observed in the confocal microscope. The serial optical sections thus obtained are conveniently analyzed as stereo projections on a laptop computer. Visualization of adherens junctions in this way allows interpretation of cell number, position and shape as well as distinguishing adradial versus interradial cells of the pharynx within a three-dimensional framework. The procedure promises to be a rapid method for comparison among species of cellular arrangements that are of phylogenetic significance and that can be coded in a matrix for phylogenetic analysis.

ENGINEERING ARABICA COFFEE FOR ROOT-KNOT NEMATODE RESISTANCE WITH CYSTEINE AND SERINE PROTEINASE INHIBITOR GENES. **Cabos, R.,[1] B. Sipes,[1] C. Nagai,[2] D. P. Schmitt,[1] H. Atkinson.[3]** [1]Plant and Environmental Protection Sciences, University of Hawaii at Manoa, 3190 Maile Way, St. John 309, Honolulu, HI 96822; [2]Hawaii Agriculture Research Center, 99–193 Aiea Heights Drive, Suite 300, Aiea, HI 96701-3911; [3]Centre for Plant Biochemistry and Biotechnology, University of Leeds, Leeds, LS29JT, UK.

Meloidogyne konaensis, the Kona coffee root-knot nematode, causes severe damage to *Coffea arabica* cv. Typica Guatemala in Hawaii. The potential of control through genetic engineering with cysteine and serine proteinase inhibitors is being explored. The construct containing the modified rice cystatin gene, OcI-D86, and a dual construct of cystatin and a cowpea trypsin inhibitor, OcI-D86/GO/CpTI, driven by a 35S or tubulin promoter have been used. Coffee somatic embryos, obtained from leaf discs, were transformed with *Agrobacterium tumefaciens* or particle gun bombardment. Secondary somatic embryos were selected with geneticin sulfate (30 mg/L G418). Plants were regenerated from 29 of 1,100 selected somatic embryo lines inoculated with *A. tumefaciens*. Fifteen plant lines were produced from the 1,200 selected somatic embryo lines from particle bombardment. Twelve of the regenerated lines tested positive for the cystatin gene with PCR analysis. Multiple plants regenerated from the same somatic embryo confirmed the presence of the transgene with 2 different primer sets. These transformed plants will be inoculated with *M. konaensis* and further evaluated in a biological greenhouse assay for resistance to the nematode.

NEMATODE AND MITE ASSOCIATES OF BARK BEETLES ON PINE IN LOUISIANA (USA) AND ON ELM IN AUSTRIA. **Carta, L. K.,[1] E. Erbe, R. Ochoa,[1] K. Klepzig, J. C. Moser,[2] H. Konrad, and T. Kirisits.[3]** [1] USDA-ARS, BARC, Beltsville, MD 20705; [2]USDA Forest Service, Southern Research Station, Pineville, LA; [3]Institute of Forest Entomology, Forest Pathology, and Forest Protection (IFFF), Department of Forest and Soil Sciences, BOKU–University of Natural Resources and Applied Life Sciences, Vienna, Austria.

Numerous adult male and female nematodes were associated with two mites in galleries of the small southern pine engraver *Ips avulsus* on loblolly pine (*Pinus taeda*) in Louisiana. The nematodes were identified as *Parasitorhabditis subelongati*, with minor morphological differences from the original description. One of the phoretic mites, *Elattoma benneti*, resided under and around the beetle legs. The other mite, *Iponemus truncatus eurus*, resided on the beetle thorax and appeared to puncture a nematode as seen when cryo-fixed under a scanning electron microscope. Until now, *P. subelongati* was reported in Russia on *Ips subelongatus*, and in the United States on *Ips calligraphus ponderosae* from northern New Mexico. We now report *P. subelongati* from a new host (*I. avulsus*) and a new location, the Southeastern United States. Additionally, on Austrian elms (*Ulmus minor*) nine phoretic mite species were associated with two elm bark beetles, *Scolytus multistriatus* and *S. pygmaeus*, which act as vectors of the Dutch elm disease fungus, *Ophiostoma novo-ulmi*. Under the elytra of both beetle species small female nematodes (285–350 micrometers) belonging to genus *Cryptaphelenchus* were observed. Larger (720–750 micrometers) adult *Neoparasitylenchus* sp. were present inside abdomens of *S. multistriatus*, but absent from *S. pygmaeus*. The beetle *S. multistriatus*, but not *S. pygmaeus*, has also been reported in the United States, Australia and New Zealand. The two nematodes appear to be new associates of the beetles. The specific identity of the nematodes, and the trophic role of the mites are presently under investigation.

CHARACTERIZATION OF SYMBIOSIS GENES IN *PHOTORHABDUS LUMINSECENS* TT01 VIA TRANSPOSON MUTAGENESIS. **Chaston, J. M., and B. J. Adams.** Department of Microbiology and Molecular Biology, Brigham Young University, Provo, UT 84602.

Symbiotic associations have developed between organisms throughout time to provide mutualistic, parasitic, or commensalistic outcomes. Two genera of nematodes, *Steinernema* and *Heterorhabditis* have developed a mutualistic association with different bacterial species in order to parasitize a wide range of insect hosts within which both the bacteria and nematode reproduce and from which they subsequently disperse. Previous studies on *Xenorhabdus* spp. hypothesize that a single gene encoding a sigma factor may be sufficient to establish symbiosis between it and its nematode symbiont, *Steinernema* spp. The mutualistic *Heterorhabditis* spp. and their partners, *Photorhabdus* spp., are typically characterized by a more specific relationship, and we expect that more than one gene is involved in their symbiotic interactions. To explore this relationship we created symbiosis-deficient *Photorhabdus luminescens* mutants by transposon mutagenesis and screened each mutant for its ability to allow growth and development of axenic *Heterorhabditis bacteriophora* TTO1. After cloning the affected region we sequenced the cloned plasmids in order to identify and characterize the affected genes.

SOIL AMENDMENTS FOR THE MANAGEMENT OF PLANT-PARASITIC NEMATODES ON PLANTAIN. **Chavarria-Carvajal, J.-A., and N. Vicente.** Department of Crop Protection, Mayagez Campus, University of Puerto Rico, P.O. Box 9030, Mayaguez, Puerto Rico 00681–9030.

Accumulation of solid wastes from human activities and agro-industries is a serious problem in Puerto Rico that represents an environment hazard and leads to significant pollution of soils, waterways, and lakes. The proper use and disposal of these materials in agricultural soils, through its application for management of phytonematodes, could be very useful to find solutions at this problem. Research was conducted to determine the effects of poultry litter for the management of plant-parasitic nematodes on plantain (*Musa acuminata* × *M. balbisiana*). The amendment was applied to nematode-infested soils at rates ranging of 0, 7.3 and 14.5 kg/plant; also, a treatment with phenamiphos at 1.5 g a.i/plant was included to determine the effectiveness of the amendment. Results showed that poultry litter was effective reducing final soil and root populations of *Radopholus similis, Meloidogyne incognita, Rotylenchulus reniformis* and *Helicotylenchus multcinctus* when compared with the chemical control. Also, the amendment improved plant development, root condition and crop yield (e.g., number of hands and fruits per bunch, and bunch weight). Poultry litter represents a suitable ecological alternative for nematode management and waste disposal in Puerto Rico.

CAENORABDITIS ELEGANS POPULATION ECOLOGY AND LIFE HISTORY. **Chen, J., and E. P. Caswell-Chen.** Department of Nematology, University of California, Davis, CA 95616.

Nematodes have a variety of life history strategies that allow them to escape stress, including mechanisms that allow escape through time or space. *Caenorabditis elegans* has life-history characteristics of a colonizing species that allows the nematode to use ephemeral resources rapidly for egg production. When food becomes limiting, facultative vivipary, a new path in its life cycle, is induced where maternal resources are used in progeny production and internal larvae consume the adult body contents to reach the long-lived, resistant dispersal stage, the dauer. Approximately 90% of lifetime egg production by unmated hermaphrodite *C. elegans* occurs during the first 3 days of adult life. Stress late in the reproductive period may not substantially reduce fitness. This hypothesis was assessed by quantitative assessment of life table and fitness parameters to compare the instantaneous rate of growth, finite rate of increase and population doubling time of worm cohorts with or without starvation. Understanding the plastic life history traits in *C. elegans* at the whole organism and molecular levels may provide insights into the survival and control of nematode pathogens of plants, humans, and animals with similar stress responses.

LYSOBACTER ENZYMOGENES AS AN ANTAGONIST OF PLANT-PARASITIC NEMATODES. **Chen, J.,[1] G. Y. Yuen,[2] D. Y. Kobayashi, [3] and E. P. Caswell-Chen. [1]** Departments of [1]Nematology, [2]Plant Pathology, and [3]Plant Biology & Pathology, [1]University of California, Davis, CA 95616; [2]University of Nebraska, Lincoln, NE 58588; and [3]Rutgers, New Brunswick, NJ 08901-8520.

The bacterium *Lysobacter enzymogenes* strain C3 produces chitinases, lipases, and proteases and so has potential as a biological control agent of plant-parasitic nematodes. We assessed the influence of *L. enzymogenes* strain C3 against the bacterial-feeding nematode *Caenorhabditis elegans*, and the plant-parasites *Heterodera schachtii* and *Meloidogyne javanica*. *Caenorabditis elegans* produced very few eggs when exposed to *L. enzymogenes* strain C3 on agar plates and 94% of hatched juveniles died after two days. *Caenorabditis elegans* produced five times fewer progeny on agar containing commercial chitinase (0.21 unit/ml) than on agar without chitinase. *Heterodera schachtii* egg hatch on an agar lawn of *L. enzymogenes* was about 50% as compared to 80% on a lawn of *E. coli*, and hatched juveniles died as the cuticle and body contents disintegrated. *Meloidogyne javanica* juveniles died after four-day exposure to a seven-day old broth culture of *L. enzymogenes*. The death and disintegration of juvenile nematodes suggests activity of proteases and lipases as has been reported for this strain. We intend to continue evaluating strain C3 for biological control of plant-parasitic nematodes.

ACTIVATION OF SYSTEMIC ACQUIRED RESISTANCE BY ACIBENZOLAR-S-METHYL AGAINST PLANT-PARASITIC NEMATODES IN *ANANAS COMOSUS*. **Chinnasri, B., B. S. Sipes, and D. P. Schmitt.** Department of Plant and Environmental Protection Sciences, University of Hawaii at Manoa, 3190 Maile Way, Honolulu, HI 96822.

Foliar application of acibenzolar-s-methyl at 100 mg/L of water to pineapple plants reduced egg production of *Rotylenchulus reniformis* and *Meloidogyne javanica* by 40% in one year. The mechanism underlying the nematode reduction indicated that acibenzolar neither caused direct toxicity to nematodes nor inhibited nematode root penetration. However, arrested nematode development and less egg deposition were observed in plants treated with acibenzolar. To determine the activation of the systemic acquired resistance in pineapple following acibenzolar application, the induction of the pathogenesis-related genes (PR) was determined using PR-1 as SAR marker. RNA was extracted from the roots of pineapple treated with 100 mg acibenzolar or water (control). First-stand cDNA was synthesized from RNA by RT-PCR using total RNA and oligo dT as the antisense primer, followed by PCR using sense and antisense degenerate primers designed from the consensus regions of PR-1 of several monocot plants. A PCR-amplified 266 bp cDNA fragment was only present in pineapple treated with acibenzolar. For sequencing, the resultant PCR product of 266 bp was ligated into a pCR®4-TOPO vector (Invitrogen) and sequenced. The BLAST program comparison showed that the nucleotide sequence of this 266 bp cDNA fragment was highly homologous to the barley basic PR-1 gene sequence. The amino acid sequence deduced from the fragment revealed 73%, 70%, 69%, 67%, 67% and 61% identity to the maize PR-1, barley PR-1, wheat PR-1, rice PR-1, tobacco PR-1, and *Arabidopsis* PR-1 genes respectively. It is likely that a PR-1 gene is expressed in pineapple treated with acibenzolar and that pineapple has a SAR pathway.

AY-9944 INHIBITS REPRODUCTION AND STEROL ISOMERIZATION IN *CAENORHABDITIS ELEGANS*. **Chitwood, David J.** USDA, ARS, Nematology Laboratory, Henry A. Wallace Beltsville Agricultural Research Center, Building 011A, Room 165B, BARC-West, Beltsville, MD 20705.

The area of sterol biochemistry represents one of the few fundamental biochemical differences between parasitic nematodes and their plant or mammalian hosts. Nematodes possess a sterol nutritional requirement, which results from their inability to biosynthesize sterols de novo. Consequently, nematodes obtain sterols from their hosts or environment and then metabolize them to other sterols better suited to functions within nematodes. AY-9944 is a compound known to inhibit various isomerases involved in the conversion of C-8(9) or C-8(14) double bonds to C-7 double bonds in plants and mammals. In this study, *Caenorhabditis elegans* was propagated in a semidefined liquid medium containing yeast extract, soy peptone, glucose, hemoglobin, sitosterol, and Tween 80; AY-9944 levels ranged from 0 to 100 ug/ml. Nematode reproduction was measured after 3 and 7 days. The results indicated that the ED_{50} was approximately 50 ug/ml. Sterols from control and inhibitor-treated nematodes were then analyzed by gas chromatography. In both cases, the predominant nematode sterol was 7-dehydrocholesterol; substantial amounts of cholesterol and lathosterol also occurred, in addition to residual dietary sitosterol. A striking difference existed in the 4-methylsterol compositions; the ratio of 4-methylsterols with a C-7(8) double bond to those with a C-8(14) double bond was 13 times larger in AY-9944-treated *C. elegans* than in controls. This difference suggests that the inhibition of reproduction in *C. elegans* could be associated with inhibition of the sterol isomerase involved in the formation of 4-methylcholest-8(14)-enol from 4-methylcholest-7-enol.

MELOIDOGYNE SPECIES WITHIN ALTERNATIVE TOMATO PRODUCTION SYSTEMS. **Church, G. T., D. Chellemi, and E. Rosskopf.** USDA, ARS, US Horticultural Research Laboratory, Fort Pierce, FL 34945.

Many *Meloidogyne* species are parasitic on tomato and when not effectively managed cause significant reductions in yield. Alternative production practices and cropping systems have the potential to manage nematode populations without the use of soil fumigation with methyl bromide. Five different land management programs were investigated for their effect on the incidence of root galling by *Meloidogyne* species. Fields were managed for three years under the following programs: conventional tomato production using soil fumigation with 1,3-dichloropropene plus chloropicrin, organic production utilizing cover crops and organic amendments, weed fallow, disk fallow, and establishment of bahiagrass pasture. In the fourth year a section of land under each management program was cultivated to tomato. Gall rating and soil samples were taken from the rhizosphere from each replicated treatment at mid-season and prior to tomato harvest. Soil collected at mid-season was placed in 13cm diameter pots and *Lycopersicon esculentum* cv. Rutgers transplants were placed in each pot. Five female root-knot nematodes were extracted from each root system and identified to species using esterase and malate dehydrogenase enzyme phenotypes. The weed fallow treatment had a significantly higher mean gall rating of 8.25 (0 to 10 scale) prior to harvest of field-grown tomatoes and *M. incognita, M. floridensis*, and *M. javanica* were identified. *Meloidogyne incognita* was identified from a single plot from the program utilizing a bahiagrass pasture prior to tomato production. *Meloidogyne floridensis* was identified from a single conventional plot. All other treatments, with the exception of the organic program, had a mean gall ratings ranging from 0.5 to 1.5. The weed fallow treatment supported a higher and more diverse population of root-knot nematodes species

AN ANALYSIS OF DEVELOPMENT OF FIELD POPULATIONS AND INBRED LINES OF *HETERODERA GLYCINES*, THE SOYBEAN CYST NEMATODE, ON RESISTANT SOYBEAN. **Colgrove, A. L., and T. L. Niblack.** Department of Crop Sciences, University of Illinois, Urbana, IL 61801.

The soybean cyst nematode is responsible for major economic losses to soybean producers. Resistant soybean varieties are used in attempts to reduce *Heterodera glycines* population densities and limit yield loss. Bioassays such as Hg-type and race tests are performed to monitor *H. glycines* population development on resistant soybeans. Assessment of virulence in field populations facilitates management decisions and increases understanding of *H. glycines* population development and soybean resistance. The lack of diversity and durability of resistant soybean genotypes are problems confronted by soybean producers. In Illinois, varieties developed from plant introduction (PI) 88788, the most commonly used source of resistance, have become less effective due to adaptation by *H. glycines* populations. Soybean lines with the highly resistant PI 437654 source of resistance should be exploited. However, some populations are now virulent on varieties developed from soybean lines such as Cyst-X (that have PI 437654 as the source of resistance). A paucity of knowledge of the nature of soybean resistance and how deployment of resistant cultivars may affect population development currently exists. Therefore, female indices from more than 1000 race and HG-type tests on field populations and inbred lines were obtained and analyzed. Initial tests indicate highly significant correlations ($p < 0.001$) for parasitism on PI 88788, PI 209332 and PI 548316 and for parasitism on PI 548402, PI 90763, and PI 89772. Previous reports indicate a similarity of resistance mechanisms in these two "groups" of soybean genotypes. As adaptation of field populations to deployed sources of resistance occurs, the effectiveness of resistant cultivars derived from soybean genotypes within these groups will be brought into question.

MUSTARD BRAN AS A BIOCHEMICAL NEMATICIDE FOR TURFGRASSES. **Crow, W. T.[1], Q. Yu,[2] and M. Chiba.[2]** [1]Entomology and Nematology Department, University of Florida, Gainesville, FL 32611;[2] Nematrol Inc., Vineland Station, Ontario, Canada L0R 2E0.

With the impending cancellation of fenamiphos, new management strategies are needed for plant-parasitic nematodes on turfgrasses. Bran derived from oriental mustard (*Brassica juncea*) releases the nematicide allyl-isothiocyanate (AITC) upon contact with water. The dry material can be added topically to turf and the AITC can be moved into the ground during irrigation. Numerous field experiments conducted over a four-year period evaluated the effectiveness of mustard bran for management of *Hoplolaimus galeatus* and *Belonolaimus longicaudatus* on turfgrasses. Multiple formulations, rates, and application methods were evaluated on several grass species. Unformulated mustard bran was bulky, caused phytotoxicity, and was difficult to apply. However, improved formulations caused no phytotoxicity, were easier to apply, and reduced population densities ($P < 0.05$) of *H. galeatus* and *B. longicaudatus* in soil. Visual improvement of turf often was pronounced ($P < 0.05$), especially in sites where *H. galeatus* was the primary nematode problem. Results of these studies indicate that formulated mustard bran could be an acceptable alternative to fenamiphos for certain turfgrass situations.

EFFECTS OF BIOLOGICAL SOIL CRUSTS ON NEMATODE COMMUNITIES IN THE ARID SOUTHWEST UNITED STATES. **Darby, B. J., and D. A. Neher.** Department of Earth, Ecology, and Environmental Sciences, University of Toledo, Toledo, OH 43606.

Biological soil crusts are key mediators of carbon and nitrogen inputs for arid land soils. Additionally, free-living nematodes are numerically and functionally important components of most soil food webs, stimulating microbial production and nutrient turnover. In this study, we test the hypothesis that biological soil crusts do affect nematode abundance and community composition. In both the Colorado Plateau, UT (cool, dry desert) and Chihuahuan Desert, NM (warm, wet desert), soils were sampled from beneath light, early-successional stage crusts dominated by cyanobacteria, or dark, late-successional stage crusts dominated by lichens and mosses. Nematodes were enumerated to genus from five replicate soil samples at 0–10 cm and 10–30 cm depth from each location and crust type combination. Nematode abundance was greater in cool UT than hot NM soils, greater from beneath dark than light crusts, and greater in shallow than deep soils. Nematode communities beneath dark crusts generally had greater generic richness, diversity, and ecological maturity than under light crusts. Canonical correspondence analysis revealed that nematode community composition discriminates first by location and secondly by crust type for UT communities and by depth for NM communities. This study demonstrates the importance of arid land biological crusts on soil microfauna and suggests that nutrient cycling and ecosystem functions are likely to be affected as well.

EARLY SEASON OXAMYL APPLICATIONS INCREASE CONTROL OF COLUMBIA ROOT-KNOT NEMATODE (*MELOIDOGYNE CHITWOODI*) IN POTATO. **David, N. L.,[1] R. E. Ingham,[1] N. D. McKinley,[2] B. A. Charlton,[3] K. J. Merrifield,[1] and N. M. Wade.[1]** [1]Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR 93771; [2]DuPont Agricultural Products, 4280 Montaigne Lane S., Salem OR 97302; [3]Ohio State University Klamath Experiment Station, 6941 Washburn Way, Klamath Falls, OR 97603.

Columbia root-knot nematode (*Meloidogyne chitwoodi*) (CRKN) is a serious pathogen of potato (*Solanum tuberosum*) in many areas of the western United States. Tuber infection by CRKN reduces tuber quality by inducing surface galling

and/or brown spots that develop around internal infection sites. Oxamyl reduces CRKN infection on potato, but a short half-life requires multiple applications at strategic times during the life cycle of the nematode for optimum control. Historically, initial oxamyl applications of 1 lb/acre have been made at 950 soil degree-days base 5 C (DD5C) after planting, coinciding with the hatch of 2nd generation juveniles, followed by applications every two weeks until potato harvest. Recent work in three climatic regions of the western United States indicates early season oxamyl applications (prior to 950 DD5C) increase CRKN control. Adding early season oxamyl applications to the standard program reduced tuber infection by 29 and 40% at Hermiston, OR in 2001 and 2002, respectively. Similarly, early season oxamyl applications reduced tuber infection by 70 and 64% in the San Luis Valley of Colorado in 2002 and 2003, respectively, and by 96% in Klamath Falls during 2002. Of the early season applications tested, in-furrow at planting was most efficacious. Population dynamics data indicate in-furrow oxamyl applications reduce the number of juveniles present in the soil during the period of initial tuber infection.

EFFECTS OF SIMULATED ACID RAIN ON NEMATODE COMMUNITIES: A GREENHOUSE EXPERIMENT. **Davidson, W. L.,[1] K. B. Nguyen,[1] R. McSorley,[1] and B. J. Adams.[2]** [1]Entomology and Nematology Department, University of Florida, Gainesville, FL 32611-0620; [2]Microbiology and Molecular Biology Department, Brigham Young University, Provo, UT 84602-5253.

As a follow-up to previous field observations, a greenhouse experiment was conducted to monitor changes of tree health and nematode communities in the presence of simulated acid rain. A randomized, complete block experiment was designed with five treatments and four replicates. Each experimental unit consisted of an 11.3-L pot with 10 kg of sandy soil and a one-year-old Florida maple, *Acer barbatum*. Over a two-month period, nitric acid, a primary component of acid rain, was added to each of the treatments in order to achieve specific pH targets. Treatment targets were pH 4.0, 3.5, 3.0, and 2.5, with 4.5 as the control. Separate soil samples were first titrated to find the amount of acid needed to bring soil pH down to these target levels. Soil pH was brought to the desired target levels and changes in the experimental units were observed. Soils were sampled at the beginning, middle, and end of the experiment. The harshest of the treatments (pH 2.5, 3.0) resulted in tree death. Nematodes present were extracted and identified. The maturity index (MI) was used as the primary tool to monitor changes in the nematode communities. The MI decreased linearly with pH, as did numbers of several key taxa. The MI was a helpful but cumbersome indicator of soil health. The *Prismatolaimus:Filenchus* ratio appeared to be a more simplified and robust indicator of soil acidification. These results confirm previous observations made from mountain forests of the Appalachians (USA), where tree death accompanied by acidified soils and low nematode MI had been documented previously.

BASE TEMPERATURE AND HEAT UNIT REQUIREMENTS FOR DEVELOPMENT OF *MELOIDOGYNE ARENARIA* AND *MELOIDOGYNE JAVANICA*. **Davila, Marisol, and D. W. Dickson.** Department of Entomology and Nematology, University of Florida, Gainesville, FL 32611.

Information on the heat units required for root-knot nematode development can provide a predictive model for their population growth. Our objectives were to determine the basal threshold temperature (Tb) for development, and heat units required for nematode development from second-stage juveniles (J2) to egg-laying females. Heat units are expressed as degree-days (DD) [(constant temperature–base temperature) × days]. To estimate the Tb and DD, freshly hatched J2 of *M. arenaria* and *M. javanica* were inoculated on okra (*Abelmoschus esculentus*) and placed in environmental control chambers at 12, 15, 18, 21, 24, 27, 30, 33, and 35 °C. Data were subjected to regression analysis to estimate the base temperature for both species. Base temperature for *M. arenaria* was estimated as 8.8 °C and that for *M. javanica* was 10.2 °C. Nematode development from J2 to egg-laying females of *M. arenaria* required 29, 23, 19, 17, 17, and 19 days at 21, 24, 27, 30, 33, and 35 °C, respectively. Development of *M. javanica* required 25, 21, 17, 17, and 19 days at 24, 27, 30, 33, and 35 °C, respectively. The heat units for development of *M. arenaria* and *M. javanica* were estimated to be 386 and 379 DD, respectively.

THE RELATIONSHIP BETWEEN CULTIVAR YIELD POTENTIAL AND PERCENTAGE YIELD LOSS TO *MELOIDOGYNE INCOGNITA* IN COTTON. **Davis, R. F.,[1] and O. L. May.[2]** [1]USDA-ARS, Crop Protection and Management Research Unit, P.O. Box 748, Tifton, GA 31793; [2]Department of Crop and Soil Science, P.O. Box 748, University of Georgia, Tifton, GA 31793.

Meloidogyne incognita causes significant yield reductions throughout the U.S. cotton belt. The term resistant means nematode reproduction is inhibited relative to a susceptible standard, whereas tolerance means that crop growth and yield are affected relatively little by nematode parasitism. Virtually all cotton cultivars on the market are susceptible to *M. incognita*, but their tolerance is not known. The amount and percentage yield suppression caused by *M. incognita* in 12 high-yielding, high-quality cotton cultivars was measured in a strip-plot study with non-fumigated and fumigated plots for each cultivar. Yield potential for each cultivar was estimated from yield in the fumigated plots. Yield suppression (kg lint/ha) ranged from 18% to 47% in 2002 and 9% to 36% in 2003, and yield potential ranged from 1484 to 2301 kg/ha in

2002 and from 926 to 1486 kg/ha in 2003. It was anticipated that the kilograms lost per hectare would increase as yield potential increased but the percentage yield loss would be similar among the cultivars. However, the percentage yield loss also differed among cultivars such that cultivars with higher yield potentials also had greater percent yield loss to *M. incognita*. Based on these data, it appears that nematode management is of greater importance in cultivars with higher yield potentials.

NEMATODE INTERACTIONS WITH AVOCADO THRIPS IN MULCHED AVOCADO ORCHARDS. **De Ley, Paul[1], K. Carter[1], M. Yoder,[1] I. Tandingan De Ley,[1] M. Brownbridge,[2] and M. Hoddle.[3]** [1]Departments of Nematology and [3]Entomology, University of California, Riverside, CA 92521; [2]Department of Entomology, University of Vermont, Burlington, VT 05405.

The avocado thrips *Scirtothrips perseae* is an invasive species capable of causing substantial losses to avocado growers in California. Application of organic mulches under avocado trees reduces emergence of adult thrips, but the mechanism(s) and agent(s) responsible for this suppression are unknown. To investigate causes of thrips mortality in mulches, we are surveying entomopathogenic nematodes, fungi, and predatory microarthropods from mulches. We report here on nematode communities encountered in mulches applied to two avocado orchards. To date, over 50 nematode species were isolated, including *Seinernema feltiae*, a known entomopathogen of western flower thrips *Frankliniella occidentalis*. *In vitro* infectivity assays of *S. feltiae* on various avocado thrips developmental stages indicate that this nematode invades and kills these insects, although the infective juveniles appear to reside only briefly inside the insect and do not necessarily release their bacterial symbionts during that time. *S. feltiae* appears to be very unevenly distributed in the sampled mulches. We also report on correlations between avocado thrips numbers and presence of other nematode species.

BIOFUMIGATION FOR ROOT-KNOT NEMATODE CONTROL IN VEGETABLE PLASTICULTURE. **Desaeger, J. A, A. S. Csinos, and K. W. Seebold.** Department of Plant Pathology, University of Georgia, CPES, Tifton, GA 31793.

Root-knot nematodes are one of the major soilborne pathogens affecting vegetables grown under plasticulture in the southeastern U.S. Due to the phasing out of methyl bromide, alternative methods to control nematodes and other soilborne pests and diseases are required. Most research has been directed toward chemical alternatives, mainly 1,3-D and metam sodium, and in the short term these appear to be the only viable alternatives to methyl bromide. Biofumigation is a term used to describe the suppression of soilborne pests and diseases by volatile degradation products produced in rotation with Brassica green manure crops. The glucosinolates contained in Brassicaceae tissues produce a variety of allelochemicals (isothiocyanates, ITC's) that are effective pesticides. The potential of biofumigation as compared to chemical fumigation was compared in a series of tests in Tifton, GA. Biofumigation, using Brassica green manure spring crops (mustard, turnip, kale, rutabaga and radish) or seed meal amendments, was less effective in controlling root-knot nematodes and soilborne fungi on a subsequent plasticulture squash crop than chemical fumigation using either methyl bromide, emulsified 1,3-D, or chemical ITC analogues such as metam sodium and dazomet. Many Brassica crops, including turnips, mustard and kale, were good hosts for root-knot nematode (*Meloidogyne incognita*), further reducing their potential to control the nematode on a following crop. The radish cv. Scarlet Globe, was a poor host to M. incognita and gave better nematode control than any of the other Brassica crops, but poorer control than metam sodium. New tests have been initiated (1) using Brassicas as winter crops instead of spring crops, including high-glucosinolate mustard cultivars, and (2) combining Brassica cover crops with low levels of chemical isothiocyanates to supplement biofumigation.

EVOLUTIONARY ANALYSIS OF HORIZONTAL GENE TRANSFER IN *MELOIDOGYNE*. **Dillman, Adler R., and B. J. Adams.** Department of Microbiology and Molecular Biology, Brigham Young University, 775 WIDB Provo, UT 84602.

Horizontal gene transfer (HGT) from prokaryotes to eukaryotes has been hypothesized to play a key role in the evolution of plant parasitism by nematodes. The mechanisms of such transfer are not well understood, and there are also many unanswered questions about how codon usage in transferred genes could maintain product function under a new genetic code. In order to explore putative HGT events we analyzed several *Meloidogyne* genes involved in plant parasitism that have been proposed as having originated through HGT events. We compared these sequences to several similar prokaryotic genes resulting from a BLASTx search in GeneBank. Our null hypothesis is that genes of similar function will be under similar selection pressure to maintain these functions in specific coding regions of the gene. Here we explore several aspects of the evolution of such HGT events by examining patterns of substitution and nucleotide composition. To identify domains under selection we used computer algorithms that analyze synonymous and non-synonymous substitution patterns in an evolutionary context. Our findings inform discussions about the role of HGT events in the parasitism of plants by nematodes.

CHARACTERIZATION OF TWO SOYBEAN CYST NEMATODE POPULATIONS THAT REPRODUCE ON PI437654 SOURCE OF RESISTANCE. **Donald, P. A.,[1] and L. D. Young.[2]** USDA ARS Crop Genetics and Production Research Unit, [1]Jackson, TN 38301 and [2]Stoneville, MS 38776.

Two soybean cyst nematode populations, LY1 and LY2, capable of reproducing on PI 437654 were identified in West Tennessee in 1995. One population was developed in the greenhouse and has a phenotype of Race 4 or HG Type 1.2.3.4.5.6.7. Reproduction was 82%, 57%, and 176% respectively on Hartwig, Anand, and 4944 (CystX) in relation to Lee 74 (PI 548658). The second population was collected from field soil and has a phenotype identical to the former population. Reproduction was 52%, 80% and 68% on Hartwig, Anand, and 4944 in relation to Lee 74 (PI 548658). Producers surveyed indicate that 15% of soybean production fields in West Tennessee were planted to Anand in 2003. In comparison, a Race 2 or HG Type 1.2.5.7 population has 1%, 14% and 0.6% reproduction on Hartwig, Anand, and 4944, respectively in relation to Lee 74. A Race 14 or HG Type 1.3.5.6.7 population had 0.4%, 7% and 3% reproduction on Hartwig, Anand, and 4944, respectively in relation to Lee 74. A Race 3 population or HG Type 0 had 0.3%, 9% and 1% reproduction on Harwig, Anand, and 4944, respectively in relation to Lee 74.

HIGH- RESOLUTION DIGITALLY MONTAGED MICROGRAPHS OF NEMATODES. **Eisenback, J. D.** Department of Plant Pathology, Physiology, and Weed Science. Virginia Tech, Blacksburg, VA 24061.

Two to thirty or more digital images of one nematode specimen photographed through a high resolution, oil immersion lens can be stitched together to form one large, highly detailed photomicrograph of the entire worm. The individual photographs are added together on different layers with image processing software in which the overlapping edges are feathered so that they merge into one seamless image. Proper alignment of the camera with the stage of the microscope, and an appropriate exposure that is used for all of the images in order to give an even brightness, are extremely important for obtaining a series of photographs that are suitable for stitching. However, the selection of a specimen that is absolutely lateral and level is even more critical. The resulting montaged images are extremely valuable for teaching and can greatly enhance the value of type and voucher specimens because these images will not deteriorate over time. They can be duplicated and recorded on CD ROM or DVD, and the viewer does not need a very expensive, high quality, research-grade microscope.

VARIABLE- RATE NEMATICIDE APPLICATIONS ON COTTON FOR RENIFORM NEMATODE MANAGEMENT. **Ellis, G. R.,[1] G. W. Lawrence,[1] S. Samson,[2] W. A. Givens,[2] and K. S. Lawrence.[3]** [1]Department of Entomology and Plant Pathology and [2]Department of GeoResources Institute, Mississippi State University, Mississippi State, MS 39762; [3]Department of Entomology and Plant Pathology, Auburn University, Auburn, AL 36849.

Field plots were established in fields naturally infested with the reniform nematode to determine the appropriate grid size to maximize the effectiveness of nematicide applications utilizing variable rate application technology. Each field plot was placed on 0.006, 0.025, 0.1, 0.2, and 0.4 ha grids and nematode samples were collected at each grid intersect. Nematode distribution maps illustrating the spatial distribution were created and analyzed for grid size effectiveness. Nematode numbers recovered from each grid determined the application rate and the number of rate changes that would occur across the field. Preliminary results indicate that the most accurate sample size for variable rate technology applications is the smaller sampling size. At this location, the 0.006 ha grid provided 93 rate changes within a one-hectare block. As the grid sample increased in size, fewer rate changes occurred. The change in application rates were 30, 14, 7.5, and sometimes 0 to 2.5 from the 0.025, 0.1, 0.2, and 0.4 ha grids, respectively. The number of rate changes is correlated with the equipment's capability to reach selected application target rates. Grid sample size will be instrumental in determining the number of sites to collect hyperspectral data. Reflectance data has the potential to replace grid sampling for the reniform nematode in cotton.

PERIODIC FLUCTUATIONS IN NUMBER OF NEW SCN CYSTS OBSERVED IN GREENHOUSE STUDIES. **Faghihi, J.,[1] R. A. Vierling,[2] and V. R. Ferris.[1]** [1]Department of Entomology, Purdue University, West Lafayette, IN 47907; [2]Indiana Crop Improvement Association and Department of Agronomy, Purdue University, West Lafayette, IN 47907.

Four soybean cyst nematode inbreds selected on Hartwig and PI 437.654, kindly made available to us by colleagues, were studied in the greenhouse over a two-year period. Dynamics of these populations were observed monthly on Williams 82, PUSCN14 (CystX®), Hartwig and PI 437.654. It was apparent that the number of new cysts (white and yellow) produced on any of these soybean lines followed an oscillating pattern. Numbers of new cysts increased to a high point, and then suddenly crashed. Following a subsequent increase, the pattern was repeated. Sometimes these fluctuations occurred gradually over several generations and appeared to be influenced by ambient temperature and day length. The changes did not appear to be host related, but were more dramatic on Williams 82. During one 6-month period, the number of new cysts increased from 60 to 103, peaked at 882, and crashed to 38. The fluctuations observed were of new cysts and do not necessarily reflect overall cyst populations in the soil.

SENTINEL NEMATODES OF ORGANIC ENRICHMENT. **Ferris, H.,[1] and T. Bongers.[2]** [1]Department of Nematology, University of California, Davis, CA 95616; [2]Laboratory of Nematology, Wageningen University, P.O. Box 8123, 6700 ES, Wageningen, The Netherlands.

The organisms of the soil food web, dependent on resources from plants or on amendment from other sources, respond characteristically to enrichment of their environment by organic matter. Primary consumers of the incoming substrate, including bacteria, fungi, herbivorous nematodes, annelids and some microarthropods, are entry-level indicators of enrichment. Quantification of abundance and biomass of this diverse group requires a plethora of extraction and assessment techniques. Bacteria, which absorb soluble organic compounds, and fungi, which degrade more recalcitrant sources, are important indicators of the origin and nature of the organic matter. Certain guilds of nematode predators of bacteria and fungi are responsive to changes in abundance of their food. Through direct herbivory, plant-feeding nematodes also contribute to food web resources. Thus, analysis of the nematode community of a single sample provides indication of carbon flow through an important herbivore channel and through channels mediated by bacteria and fungi. Some nematode guilds are more responsive than are others to resource enrichment. Generally, those nematodes with short lifecycles and high reproductive potential most closely mirror the bloom of bacteria or respond most rapidly to active plant growth. The feeding habits of some groups remain unclear. For example, nematodes of the Tylenchidae may constitute 30% or greater of the individuals in a soil sample; further study will be necessary to determine which resource channels they portray and the appropriate level of taxonomic resolution for this group. A graphic representation of nematode indicators, based on their biomass and metabolic activity, may be a useful tool for assessing the importance of the bacterial, fungal and herbivore resource channels in an extant food web.

MORE TAXA OR MORE GENES?–WHICH IS BETTER? **Ferris, V. R.,[1] A. Sabo,[2] J. G. Baldwin,[3] M. Mundo-Ocampo,[3] R. N. Inserra,[4] S. Sharma.[5]** [1]Department of Entomology, Purdue University, West Lafayette, IN 47907; [2]Genome Sequencing Center, Washington University, St. Louis, MO 63108; [3]Department of Nematology, University of California, Riverside, CA 92521; [4]Florida Department of Agriculture, DPI, Nematology, P.O. Box 147100, Gainesville, FL 32614; [5]Department of Agriculture, Baron Hay Court, South Perth, Western Australia.

For phylogenetic analyses utilizing molecular data, is it better to use more taxa or more genes? We initially obtained and analyzed DNA sequence for about 600 base pairs (bp) of the ribosomal RNA gene (rDNA) and the ITS rDNA (about 1000 bp) for a small group of five heteroderoid taxa for which little or no molecular data were previously available. These taxa were: *Afenestrata koreana, Ekphymatodera thomasoni, Bilobodera flexa, Cactodera betulae*, and *Heterodera bifenestra*. We then increased the number of species to eleven. We analyzed the separate and combined 18S and ITS rDNA for both sets of species. The phylogenetic relationships among the larger group of taxa, using the concatenated 18S and ITS data, were more similar to those of the five taxa based on the analysis of ITS rDNA data than on the analysis of the five taxa with 18S data alone. One of the two noncyst-forming species, *E. thomasoni*, grouped with cyst-forming species of Heteroderoidea, whereas a second noncyst-forming species, *B. flexa*, was separated by a long branch. *Afenestrata koreana*, with a weakly sclerotized cyst, grouped closely with *H. bifenestra*. The nature of the taxa analyzed was more important than the sheer number of taxa or genes used. Our observations may have implications for our understanding of the evolution of cyst formation, including the possibility of secondary loss of the cyst in heteroderoids.

COMPARISON OF FENWICK CAN AND SCHUILING CENTRIFUGE EXTRACTION OF CYSTS OF THE SUGAR BEET NEMATODE *HETERODERA SCHACHTII*. **Fischer, R., and J. O. Becker.** Department of Nematology, University of California, Riverside, CA 92521.

The Fenwick can extraction is perhaps the most frequently used method for cyst extraction and is based on a combination of flotation and sieving. The Schuiling centrifuge is a semi-automatic flotation/centrifugation method that has been primarily used in The Netherlands for the extraction of potato cyst nematodes. Both methods were compared in their efficacy and time requirement for extracting *H. schachtii* cysts from inoculated as well as naturally infested field soil of both high and low soil moisture. Among the inoculated samples no significant differences were observed in the number of recovered cysts in regard to the method of extraction, the soil type or the soil moisture. A higher rate of cyst recovery was found for the Schuiling method for moist samples of naturally infested soil. The Schuiling centrifuge method required only 1/4 of the time that was needed for the Fenwick can extraction.

NEMATODE COMMUNITY STRUCTURE: AN INDICATOR OF MANAGEMENT-INDUCED CHANGES IN NUTRIENT FLUXES AND INTEGRITY OF THE SOIL FOOD WEB IN TREE-FRUIT PRODUCTION SYSTEMS. **Forge, T.,[1] D. Granatstein,[2] E. Hogue,[3] G. Neilsen,[3] and D. Neilsen.[3]** [1]Agriculture and Agri-Food Canada, Pacific Agri-Food Research Centre, Agassiz, BC V0M 1A0; [2]Center for Sustaining Agriculture and Natural Resources, Washington State University, Wenatchee, WA; [3]Agriculture and Agri-Food Canada, Pacific Agri-Food Research Centre, Summerland, BC V0H 1Z0.

A series of field experiments have been established in British Columbia and Washington State to evaluate the impacts of alternative orchard floor management strategies on tree growth, weed management, nutrient dynamics and biological interactions in root zone soil of high-density apple production systems. Treatments considered to date include municipal composts, dairy manure solids, shredded office paper and alfalfa hay, as well as several combination treatments such as

shredded paper applied over municipal compost. The control in all experiments was the conventional practice of controlling orchard floor vegetation with glyphosate. Impacts of the various treatments on structure and function of the soil food web were assessed through analyses of the structure of communities of free-living nematodes, with particular reference to the Enrichment Index (EI), Channel Index (CI) and Structure Index (SI). In general, nematode community indices were very responsive to changes in orchard floor management, discriminating among treatments in all experiments. The EI was increased, relative to the control, under treatments expected to result in increased nutrient fluxes, such as combinations of shredded paper mulch applied over municipal compost and alfalfa hay applied over dairy manure solids. Ongoing research is attempting to correlate the EI with measurements of actual fluxes of N and P in the root zone. The SI was not consistently affected by organic mulch treatments. Population growth of the root-lesion nematode, *Pratylenchus penetrans*, was reduced by organic mulch treatments in two of the four experiments where it was present.

CHARACTERIZATION OF THE EFFECTS OF CHILE PEPPER, YELLOW NUTSEDGE, AND PURPLE NUTSEDGE ON *MELOIDOGYNE INCOGNITA* REPRODUCTION. **Fuchs, J.,[1] S. H. Thomas,[1] J. Schroeder,[1] L. W. Murray.[2]** [1]Department of Entomology, Plant Pathology, and Weed Science and [2]University Statistics Center, New Mexico State University, Las Cruces, NM 88003.

Greenhouse studies were conducted in 2000 and 2001 to determine if yellow nutsedge (*Cyperus esculentus*, YNS), purple nutsedge (*C. rotundus*, PNS) and chile pepper (*Capsicum annuum* cv. NM 6-4), grown individually and competitively, influence the level of infection, rate of reproduction, or life cycle of *M. incognita*. Pots of all plant treatments (chile, YNS, and PNS alone, chile+YNS, chile+PNS) were inoculated with 5,000 *M. incognita* eggs and harvested at 3-4 day intervals beginning 21-days post inoculation and continuing through day 45. Eggs and egg masses per gram of dry root and eggs per egg mass were measured for each plant at each sample date. No egg masses occurred on rhizome or tuber surfaces of either nutsedge species. There were more *M. incognita* females per gram of root and more eggs per female on chile than on either nutsedge. Effects of competition from chile on either nutsedge species did not affect the overall nematode reproduction on nutsedge but tended to increase the number of eggs produced per egg mass. However, competition from nutsedge on chile reduced overall reproduction on chile but had no effect on eggs per egg mass. Initiation of nematode reproduction among host plant treatments was similar. Overall quantity and rate of *M. incognita* reproduction was greater on chile than on either nutsedge species. However, the quantity of nutsedge root biomass available for *M. incognita* reproduction and tubers that are overwintering reservoirs for *M. incognita* make both YNS and PNS important hosts for maintaining *M. incognita* populations in the field.

QUANTIFICATION OF *FUSARIUM SOLANI* F. SP. *GLYCINES* IN SOYBEAN ROOTS AND *HETERODERA GLYCINES* CYSTS WITH REAL-TIME QUANTITATIVE POLYMERASE CHAIN REACTION. **Gao X.,[1] T. A. Jackson,[1] K. N. Lambert,[1] S. Li,[1] G. L. Hartman[2] and T. L. Niblack.[1]** [1] Department of Crop Sciences, University of Illinois, Urbana, IL 61801; [2] USDA-ARS, Urbana, IL 61801.

Soybean sudden death syndrome (SDS) caused by *Fusarium solani* f. sp. *glycines* and soybean cyst nematode (SCN, *Heterodera glycines*) inflict significant soybean yield loss in the U.S. Although either pathogen can infect soybean roots alone, foliar symptoms of SDS may be more severe when both pathogens are present. The nature of this interaction is poorly understood. To investigate the impact of SCN on SDS, a real-time quantitative polymerase chain reaction (QPCR) assay was developed for quantification of *F. solani* f. sp. *glycines* relative to soybean DNA. Relative amounts of fungal DNA were quantified based on detection of the fungal mitochondrial small subunit rRNA gene and the soybean cyclophilin gene. Relative quantities of *F. solani* f. sp. *glycines* were determined in fresh and dry root samples. In addition, *F. solani* f. sp. *glycines* was detected and quantified from SCN cysts. With the absolute QPCR assay, DNA of *F. solani* f. sp. *glycines* was detected in quantities as low as 9.0×10^{-5} ng, equivalent to or lower than the fungal DNA content in one cyst. The QPCR assay is reliable if care is taken to avoid reaction inhibition and may be used to investigate the interaction between SCN and *F. solani* f. sp. *glycines*. This is the first report of relative QPCR using the comparative threshold cycle (Ct) method to quantify the DNA of a plant pathogen relative to its host DNA.

GENETIC DIVERSITY AMONG NON-PATHOGENIC *FUSARIUM OXYSPORUM* STRAINS FROM A BEET CYST NEMATODE SUPPRESSIVE SOIL. **Gao, X.,[1] B. Yin,[2] J. Borneman,[2] and J. O. Becker.[1]** Departments of [1]Nematology and [2]Plant Pathology, University of California, Riverside, CA 92521.

Fusarium oxysporum strains were among the most commonly isolated fungi from cysts and eggs of *Heterodera schachtii* obtained from a beet cyst nematode-suppressive soil. Twenty-six strains of the fungus differed substantially in colony appearance and metabolite production on solid culture media. Phylogenic analysis of their rRNA ITS nucleotide sequences revealed considerable genetic variability. Seven representative strains were individually evaluated for their potential to parasitize beet cyst nematode eggs and to cause *H. schachtii* population suppression. Each test strain was introduced into fumigated soil at a propagule density similar to that of *F. oxysporum* in suppressive soil (10^3 cfu/cc soil). The suppressive soil and its fumigated equivalent served as controls. The nine treatments were filled into 1500 cc pots that were arranged in a randomized complete block with four replications. A four-week-old Swiss chard seedling was transplanted into each

pot that was infested with 5000 second-stage juveniles of *H. schachtii*. After 1180 degree days in the greenhouse, all *Fusarium* strains parasitized *H. schachtii* eggs, but at considerably different levels. While one strain parasitized less than 10% of the eggs, the most aggressive strain was found in approximately 35% of the eggs. This degree of parasitism was not significantly different from the one observed in the suppressive soil. However, in contrast to the originally suppressive soil, in none of the *Fusarium*-infested soils was the population of *H. schachtii* significantly reduced after two nematode generations.

MOLECULAR PROFILING OF RHIZOSPHERE BACTERIA THAT CAN SUPPRESS PLANT-PATHOGENIC NEMATODES. **Gardener, B. Mc.** Department of Plant Pathology, Ohio State University–OARDC, Wooster, OH 44691.

In any given soil, one or more microbial populations may inhibit the activities of plant pathogenic nematodes. Molecular profiling methods can be used to dissect the relative contributions of diverse microbial populations to complex ecological phenomena such as plant disease suppression. These methods typically involve comparisons of the abundance of multiple DNA and/or RNA sequences present in different samples. Different approaches to profiling these diverse sequences may be used to quantify their relative abundance among samples. Correlations between the amount of disease developing in different samples and the abundance of defined sequences are then used to identify ecologically-important populations of microbes. Subsequent use of more specific probes can provide independent confirmation of population shifts observed in profiles generated with more general probes. Because of the high-throughput nature of such techniques, molecular profiling can now be used to further establish the biogeographical range of ecologically significant microbial populations and their contributions to plant health.

REDIFFERENTIATION OF PLANT ROOTS BY SEDENTARY NEMATODES: UNRAVELING THE MOLECULAR MECHANISMS. **Gheysen, G.** Department of Molecular Biotechnology, Faculty of Agricultural and Applied Biological Sciences, Coupure links 653, B-9000 Gent, Belgium.

Sedentary endoparasitic nematodes are an important problem in agriculture and their complex relationship with the plant host is still not very well understood. From a scientific viewpoint the ability of these sedentary nematodes to induce specialized feeding sites in plant roots is one of the most fascinating aspects of this plant-parasite interaction. Cyst nematodes generate syncytia by cell wall degradation and root-knot nematodes induce giant cells by mitosis without cytokinesis. In the initial phases of our project, the emphasis was on the identification of plant genes that are upregulated by nematode infection, especially during the early phases of the interaction. Now that many different upregulated genes have been isolated, the focus is on the characterisation of specific examples. One of the early upregulated plant genes in syncytia and giant cell is an auxin-inducible transcription factor from the WRKY family. We have constructed plants overexpressing or producing RNAi for this transcription factor. They develop fairly normal except for some effects on root development indicating a common step in root and feeding site development. Nematode infection assays are being performed. An important question in the plant-nematode interaction is how these changes in gene expression and cell differentiation are induced by the nematode. Several strategies are being employed to characterise the nematode pharyngeal gland secretions. We are currently analysing the possible role of a secreted ubiquitin-extension protein in the plant-nematode interaction by trying RNAi on *Heterodera schachtii* , the beet cyst nematode and by analysis of transgenic plants. Transgenic plants have been constructed that express the ubiquitin expression protein as a fusion with gfp with a nucleolar localisation as result. We are currently analysing possible effects of this protein on plant cells.

ISOLATION OF A *PASTEURIA* SP. THAT IS EASILY CULTURED ON A BACTERIOVOROUS NEMATODE, *BURSILLA* SP. IN SOIL. **Giblin-Davis, R. M.,[1] B. J. Center,[1] D. S. Williams,[2] L. M. Schmidt,[2] J. A. Brito,[3] D. W. Dickson,[4] and J. F. Preston.[2]** [1]University of Florida-IFAS, Fort Lauderdale Research and Education Center, 3205 College Ave., Davie, Florida, 33314-7799; [2]Microbiology and Cell Science, University of Florida-IFAS, P.O. Box 110700, Gainesville, FL 32611-0700; [3]Division of Plant Industry, P. O. Box 147100, Gainesville, FL 32614-7100; and [4]Department of Entomology and Nematology, University of Florida-IFAS, P.O. Box 110620, Gainesville, FL 32611-0620.

A putative *Pasteuria* species, strain R-1, which is an obligately endoparasitic gram-positive prokaryote that parasitizes a bacterial feeding nematode, *Bursilla* sp. was discovered in bermudagrass (*Cynodon* sp.) field plots at the Fort Lauderdale Research and Education Center in Davie, FL. To isolate and build up the strain of R-1 *Pasteuria* and create a suppressive soil, the *Bursilla* sp. was first isolated from the field and cultured xenically on tryptic soy broth agar. Next, cultured populations of un-infested nematodes (>100,000) were inoculated into 8 g soil aliquots from a marked transect through the area and examined after 72 hrs for spore attachment. Areas that were positive for R-1 encumbered *Bursilla* sp. were used to create a suppressive soil by continuously adding >500,000 *Bursilla* sp. every two weeks for greater than seven months. Bioassay of this suppressive soil with un-infested *Bursilla* sp. yielded 80% of nematodes of all stages (except the egg) that were heavily encumbered with >18 R-1 endospores within 7 days of exposure. Hemocoelic microcolonies and endospore-filled nematodes were recovered starting about one week after exposure. R-1 was distinguished from other described species of *Pasteuria* using ultrastructure of the mature endospores and comparisons of genes encoding 16S rRNA and sigE.

Development and sporogenesis is similar to other nematode-specific *Pasteuria*. This is the first report of sustained *in vivo* culture of an isolate of *Pasteuria* on a bacteriovorus nematode.

NATURAL PRODUCTS FOR MANAGEMENT OF LESION NEMATODE, *PRATYLENCHUS PENETRANS* ON EASTER LILY. **Giraud, D. D.,[1] B. B.Westerdahl,[2] L. J. Riddle,[3] C. E. Anderson,[2] and A. Pryor.[4]** [1] University of California Cooperative Extension, Eureka, CA 95503; [2] Dept. of Nematology, University of California, Davis, CA 95616; [3] Easter Lily Research Foundation, Brookings, OR 97415; [4] SoilZone, Inc., Davis, CA 95616.

Easter lily bulbs for greenhouse forcing are propagated in Del Norte County, CA and Curry County, OR. *P. penetrans* infestation of soil and roots is a serious detriment to production. In two years of field trials, natural products were tested alone, and in combination with chemical standards and compared to untreated controls. Each treatment consisted of three replicates in a randomized complete block design. Several of the treatments containing natural products such as DiTera, and *Trichoderma* reduced nematode populations within roots or substantially improved growth of bulbs.

TOWARD A HIGH-THROUGHPUT MOLECULAR ANALYSIS OF NEMATODE COMMUNITIES. **Griffiths B., T. Daniell, S. Donn, and R. Neilson.** Plant-Soil Interface Programme, Scottish Crop Research Institute, Dundee, DD2 5DA, Scotland.

Given the inexorable rise in numbers of 18S rDNA sequences from nematode species placed on publicly available databases, together with recent advances in nematode community analysis and interpretation, it was our intention to combine the two areas and explore the possibility of using molecular analysis of nematode communities to report on community composition. Nematodes were extracted from an arable field at SCRI by sieving and decanting, combining the extracts from the coarse and fine sieves. The nematode samples were split into two, half for counting and morphological identification and half for molecular analysis. DNA was extracted, PCR amplified and cloned. Full length 18S sequence was generated from 224 clones, which could be ascribed to 35 different types (rarefaction analysis confirmed that we had exhausted the number of clone types) which were compared with existing database types on a phylogenetic tree. When compared with results of the morphological analysis, the clone data matched poorly with numerical data. Thus, Rhabditida represented 30% of total numbers but only 2% of clones, while Mononchida were 4% of total numbers and 33% of clones. However, there was a much better match between clone number and nematode biovolumes, which were 4% and 35% respectively. These results suggest the potential of a high throughput molecular approach, but the results also gave low clone numbers from certain taxa (e.g. Rhabditida, Tylenchida) indicating that DNA extraction and PCR amplification efficiency from a range of selected taxa should be confirmed. Results of these tests will also be presented.

COMPARATIVE PERFORMANCE OF DIFFERENT RATES OF VAPAM AND KAPAM FOR *MELOIDOGYNE CHITWOODI* MANAGEMENT IN POTATOES. **Hafez, S. L., and P. Sundararaj.** University of Idaho, Parma Research and Extension Center, 29603 U of I Ln, Parma, Idaho 83660.

Experiments were conducted to compare the efficacy of different rates of Vapam to an untreated check for the control of Columbia root knot nematode in a potato field. A field with indigenous population of Columbia root-knot nematode was selected. Experimental design was a randomized complete block design with five and four treatments of five replications each for the first and second experiment respectively, including an untreated check. Different rates of Vapam and Kapam were applied to a depth of 8–10″ by fumigation bar. Potato seed pieces were planted in rows three feet apart. At maturity potato was harvested and yield, quality, and nematode infestation was determined after 60 days of storage. In both experiments, application of all rates of Vapam and Kapam treatments significantly reduced the root knot nematode infected potato tubers as compared to untreated control. The percent nematode infection in the first experiment ranged from 0.0–31.9. Lowest level of nematode infection was observed in the Vapam treatment of 40 gal/A. In general, all treatments significantly increased the marketable and total yield of potato tubers as compared to control. In the second experiment, lowest level of nematode infection was observed in the plots treated with Vapam 80 gal/A. Nematode infection ranged from 8.1 to 21.3 in the treated plots as compared to 91.6 % in the control plot. However there is no significant difference in the reduction of percent nematode infection among the treatment rates applied.

EVALUATION OF CHEMICAL NEMATICIDES FOR THE MANGEMENT OF *MELOIDOGYNE CHITWOODI* AND *PARATRICHODORUS* SPP. ON POTATO IN IDAHO. **Hafez, S. L., and P. Sundararaj.** University of Idaho, Parma Research and Extension Center, 29603 U of I Ln, Parma, Idaho 83660.

Three field experiments were conducted in Idaho to determine if Temik alone or along with Mocap is effective in reducing the *Meloidogyne chitwoodi* or Temik and Fosthiazate were effective on *Paratrichodorus* spp. in a potato field. For all experiments the experimental design was a randomized block with six replications of plot size 15 × 50 ft planted with the potato cultivar 'Russet Burbank'. Mocap was surface broadcast using a hand held sprayer and Temik was applied in a 4–6″ band at planting in front of the planter shoe. Data indicated that application of Mocap with Temik significantly increased the total yield of tubers as compared to untreated check. Percent infection reduced to 4.6% as a result of

treatments. For the second experiment, the treatments were Mocap alone or with Temik and an untreated control. Yield increase was more by Mocap + Temik treatment than Mocap alone. Percent root knot nematode infested tubers reduced to 60.3 and 25.8 by Mocap and Mocap + Temik respectively as compared to control (95%). In the stubby root nematode management experiment, Temik was applied at planting in the furrow with seed pieces, Fosthiozate was surface broadcast using a hand held sprayer and Agrimerk was pre-plant incorporated with disc. Yield of potato tubers under different treatments indicated that there is an increase in marketable yield by the different combination of Temik and Fosthiozate as compared to control plot. Percent infection of tobacco rattle virus which is transmitted by stubby root nematode ranges from 10.0 to 35.0. Lowest level of infection was observed in the treatment of 20lbs/A Temik.

COMPARISON OF TOTAL SOLUBLE PROTEIN PROFILE OF *PRATYLENCHUS LOOSI* (THE LESION NEMATODE) POPULATIONS FROM VARIOUS TEA- GROWING REGIONS IN GILAN PROVINCE, NORTHERN IRAN. **Hajieghrari, B., M. Mohammadi, A. Kheiri, Z. Tanhamaafi.** Department of Plant Pathology, College of Agriculture, University of Tehran, Aemamzade Hasan Karaj, Tehran, Iran. *Pratylenchus loosi* , the lesion nematode, is one of the most devastating and dangerous pest of *Camellia sinensis* (L.) O. Kuntze in tea growing regions of the world. In addition to tea, the P. loosi also attacks apple, citrus and pear trees. SDS-PAGE has been widely used to analyze and compare soluble protein patterns among nematode species, biotypes and populations. In this study, a total of 16 populations of *P. loosi* were collected from various infected tea growing areas in Gilan province, Iran. Nematodes were extracted from root tissue and soil surrounding the roots of infected tea plants. *P. loosi* was identified using the identification keys described by Handoo and Golden (1989) and Fredrick and Targan (1989). A total of 40 nematodes from each population representing different larval stages and mature were sonicated in 20 ìl 1× sample buffer and loaded into 5% stacking gel. Proteins were resolved in 12% gel and stained using silver stain method. The results showed that protein banding pattern was similar among *P. loosi* populations and differences were insignificant.

NEMATICIDAL ACTIVITY IN FREEZE-DRIED NASTURTIUM TISSUE. **Halbrendt, J.** Penn State Fruit Research and Extension Center, Biglerville, PA 17307.

Six varieties of Nasturtium (*Tropaeolum majus*) including Peach Melva, Moonlight, Jewel Mix, Vesuvius, Alaska and one unknown variety and Sorghum Sudan grass (*Sorghum sudanense*) (cv. Piper) were grown to maturity in *Xiphinema americanum* infested field plots. Nematode counts at the beginning and end of the season indicated that all plants were good hosts. Samples of leaf and root tissues were quick frozen to −80 F and the remaining plant material was incorporated back into the plots as a green manure on Sept 10. Nematode counts taken two weeks after incorporation showed populations were reduced to approximately 50% of the pre-incorporation level in all treatments. The frozen tissue was pulverized and mixed with dry sterile sand in concentrations that ranged from 0.5–5.0 mg/cm^3 sand. Dagger nematodes were hand picked into 2-ml sample cups containing 250 µl sterile distilled water and 1.5 cm^3 of the plant/sand mix was added. The cups were sealed and incubated for 24 hours at 24 C. All nematodes were recovered and the numbers of alive and dead nematodes were analyzed by probit analysis. All Nasturtium tissues were highly toxic to dagger nematodes with LC50 values that ranged from 0.6 to 1.3 mg/cm^3 for leaf tissue and 0.8 to 3.45 mg/cm^3 for root tissue. None of the Sudan grass tissues were toxic at the levels tested.

AN ANTIMICROBIAL PEPTIDE FROM THE FLESH FLY CONFERS RESISTANCE TO THE ROOT-KNOT NEMATODE (*MELOIDOGYNE INCOGNITA*). **Hamamouch, N., J. D. Eisenback, and J. Westwood.** Department of Plant Pathology, Physiology, and Weed Science, Virginia Tech. Blacksburg , VA 24061.

Sarcotoxin IA is a bactericidal peptide from the flesh fly (*Sarcophaga peregrina* Robineau-Desvoidy, 1830). The gene encoding this toxin was fused to a promoter taken from a defense related isogene of 3-hydroxy-methylglutaryl coenzyme A reductase (*HMG2*). The *HMG2* gene is expressed specifically at the site of the nematode feeding, thereby controlling spatial and temporal accumulation of the toxin. The sarcotoxin IA gene construct was introduced into tobacco (*Nicotiana tabacum* L.) using an *Agrobacetrium*-mediated transformation. Integration of the transgene into the plant genome and expression was confirmed by PCR and RT-PCR, respectively. Transformed plants were challenged with 5,000 eggs of the root-knot nematode (*Meloidogyne incognita* (Kofoid and White, 1919) Chitwood, 1949 race1) and the number of galls was counted after 54 days. Tobacco plants expressing the peptide exhibited normal growth and development. Moreover, sarcotoxin IA significantly reduced nematode infection by nearly 50% in the transformed plants as compared to the non-transformed plants.

POPULATION DYNAMICS OF BELONOLAIMUS LONGICAUDATUS COMMERCIAL STRAWBERRY FIELDS IN FLORIDA. **Hamill, J. E., and D. W. Dickson.** Department of Entomology and Nematology, University of Florida, Gainesville, FL 32611.

The sting nematode, Belonolaimus longicaudatus, is a highly virulent, aggressive pathogen of strawberry, Fragaria X ananassa, grown during the winter months (October–March) in Dover, FL. Disease problems associated with sting

nematode on strawberry are unusual in that all commercial strawberry farms in this region fumigate the soil with methyl bromide before planting. Studies were initiated in September of 2002 (one farm), and January 2003 (two farms) to determine the population dynamics and vertical distribution of this pathogen during the strawberry growing season. A bucket auger (13-cm deep × 10 cm diameter) was used to take soil cores monthly at six depths; 0 to 13, 13 to 26, 26 to 39, 39 to 53, 53 to 66, and 66 to 79. Nematodes were extracted from each 100 cm3 soil sample using a centrifugal-flotation method. Soil temperature was recorded over the season and volumetric soil moisture was taken from each sample. Nematode numbers from samples taken during the summer months (June to September) were not significantly different at depths sampled. However, nematode numbers from the 0 to 13 cm and 13 to 26 cm depths increased rapidly after strawberry planting in October and peaked in the 0 to 13 cm depth at 500 to 700 nematodes/100 cm3 of soil during the month of January. Nematode numbers began to decrease rapidly after January and eventually stabilized at all depths sampled by June. The decrease of the nematode population after January appears to be related to food source and not temperature or moisture. If a susceptible crop is planted after strawberry, sting nematode population densities in the 0 to 13 cm depth will increase.

MORPHOLOGICAL CHARACTERIZATION OF A NEW ROOT-KNOT NEMATODE (*MELOIDOGYNE* SP.) ON GINGER FROM THAILAND. **Handoo, Z. A, L. K. Carta, and A. M. Skantar.** USDA, ARS, Nematology Laboratory, Beltsville, MD 20705.

In October 2002, a root-knot nematode was discovered on roots of Ginger (Zingiber spp.) that was intercepted by APHIS at San Francisco port from Thailand and was identified as representing an undescribed *Meloidogyne* sp., based on morphological observations. The importer of the Zingiberaceae from Thailand provided the following information: The plants were bought at a local Bangkok market supplied by local nursery growers and the plants were a variegated variety of Alpinia sp. or Zingiber sp. The roots exhibited symptoms of galls typical of root-knot nematode. Heavily infected roots were dark brown to black-colored, and from each infected root area we recovered clusters of 1–4 root-knot nematode females with egg masses attached. All the life stages of this species (juveniles, males and females) were heavily attacked by *Pasteuria* sp. spores. Comparison with three other morphologically related species, *M. incognita, M. arenaria*, and *M. megatyla*, revealed that the new species differs from these by one or more of the following: body and tail length, shape of head, tail and tail terminus of second-stage juveniles; stylet length and shape of spicules in males; and in the female stylet length, shape of knobs, and distinctive perineal pattern. This pattern is smooth with coarse striae, dorsal arch is high and sometimes rectangular; and striae in and around the anal area form a thick network-like structure interrupted by a prominent lateral line. Second-stage juveniles have a long slender tail with inflated rectum and long gradually tapering hyaline tail part, ending in rounded terminus. Male spicules have a bidentate terminus. Additional information regarding distribution of this nematode within the region is needed, especially in fields throughout Thailand.

GENE EXPRESSION IN GIANT CELLS: ANALYSIS BY LCM AND RT-REAL TIME PCR. **He, Bin, C. W. Magill, and J. L. Starr.** Department of Plant Pathology & Microbiology, Texas A&M University, College Station, TX 77840.

Giant cells are enlarged multinuclear cells induced by root-knot nematodes in susceptible host roots that function to provide nutrients to nematode. A major obstacle to the study of giant cells is that of collecting tissue samples that are specific to giant cells. Laser capture microdissection (LCM) is a technique that allows one to sample a single giant cell. A focused laser beam was used to collect a sample of giant cell cytoplasm from fixed and sectioned tissues. RNA was extracted from those isolated samples using TRI REAGENT. The sample was homogenized and lysed in TRI REAGENT and the homogenate was separated into aqueous and organic phases by chloroform addition and centrifugation. RNA remains exclusively in the aqueous phase, DNA in the interphase, and proteins in the organic phase. This technique avoids most of DNA contamination in RNA sample. Using the tomato gene *Rb7* as a test sample, specific primers were designed to measure level of *Rb7* by Real-Time PCR. This procedure will allow us to quantitate expression of genes of interest at different stages of giant cell development without contamination from surrounding cells.

SPORULATION OF *PASTEURIA PENETRANS* INHIBITED IN AN ISOLATE OF *MELOIDOGYNE ARENARIA*. **Hewlett, T.,[1] R. Mulrooney,[2] K. Smith,[1] and S. Griswold.[1]** [1]Pasteuria Bioscience LLC, Alachua, FL 32615;[2] Department of Plant and Soil Sciences, University of Delaware, Newark, DE 19716-2170.

A population of *Meloidogyne arenaria* was collected from Longwood Gardens (LG),PA, reared on 'Rutgers' tomato, and subsequently infected with *Pasteuria penetrans* isolate E1. This P. penetrans isolate was collected from and regularly cultured on *M. arenaria* from Alachua, Fl. Mycelial balls are present at 220 degree days (DD) and sporulation is complete by 560 DD in this population. In the LG population, typical vegetative cell growth was detected at 220 DD, but mycelial ball formation and sporulation did not occur after 560 DD. Vegetative cells collected from this isolate were placed in E1 growth media and within three to four days mycelial ball formation began and after14 days some spores were produced. Little is known of the sporulation factors for *P. penetrans* but it is assumed to be similar to *Bacillus* species. This study

indicates that nutritional factors lacking in the LG population of *M. arenaria*, but present in the Alachua population and Pasteuria Bioscience growth media, are required for sporulation of the E1 isolate of *P. penetrans*.

ANALYSIS OF ENTOMOPATHOGENIC NEMATODE DISTRIBUTIONS IN FLORIDA VIA GIS MAPPING. **Hilton, L., and B. J. Adams.** Microbiology and Molecular Biology Department, Brigham Young University, Provo, UT 84602.

Studies on the efficacy and persistence of entomopathogenic nematodes (EPNs) in agricultural ecosystems have shown that although efficacy may be similar, persistence varies by ecosystem. We are interested in exploring geophysical characteristics associated with the distribution of indigenous EPNs. Under the assumption that the distribution of indigenous EPNs may inform predictions about which species or strains are associated with geophysical parameters, such as soil biogeochemistry, we analyzed surveyed survey data from citrus growing areas in Florida for EPNs. Nematode distributions were plotted using ArcView GIS on geophysical maps. Correlations between the distribution of EPNs and geophysical parameters are explored in the context of explaining and predicting patterns of efficacy and persistence of EPNs in Florida citrus agroecosystems.

BACTERIAL PATHOGENICITY AND INNATE IMMUNE RESPONSES IN *CAENORHABDITIS ELEGANS.* **Hodgkin, J.** Department of Biochemistry, University of Oxford, Oxford OX1 3QU, UK.

Recent investigations in a number of laboratories (notably those of Ausubel, Aroian, Ewbank and Darby) have used *C. elegans* to investigate the interaction between pathogenic bacteria and nematodes. Evidence for a variety of specific host responses has been obtained, indicating that nematodes have a flexible repertoire of innate immune responses to bacterial infection. Our research has concentrated mainly on a novel pathogen of *C. elegans* called *Microbacterium nematophilum*, which can establish a rectal infection by tight cuticular adherence and induces conspicuous local swelling of rectal tissues. Numerous mutants altered in the response to *M. nematophilum* have been isolated and studied. Infection leads to activation of a protective ERK MAP kinase cascade which causes rectal swelling. Most mutants resistant to infection are defective in pathogen adherence and many exhibit altered cuticle properties. Molecular investigation of the corresponding genes is providing information on innate immune responses and cuticle structure.

ULTRASTRUCTURAL ANALYSIS OF THE FEEDING SYSTEM IN THE POTATO CYST NEMATODE, *GLOBODERA PALLIDA*. **Holland, C.,[1] G. Brennan,[1] C. Felming,[2 3] and N. Marks. [1]** [1]Parasitology Research Group; [2]School of Agriculture and Food Science, Queen's University Belfast, BT9 7BL; [3]Department of Agriculture and Rural Development Northern Ireland, Newforge Lane, Belfast, BT9 5PX.

Globodera pallida penetrate the root system of potato plants and establish feeding sites within the root. Feeding activities are dependant upon the stylet and the secretion of products from dorsal and ventral secretory glands. Gland products accumulate in distinct reservoirs which open via valves that are believed to be under neuronal control. Electron microscopic analyses of the anterior neuromuscular system of *G. pallida* J2s were used to examine the interrelationships of nerve and muscle associated with the feeding apparatus. Following completion of the basic ultrastructural analyses, electron immunocytochemistry and confocal scanning laser microscopy were employed to investigate the distribution and expression of FMRFamide-related neuropeptides (FaRPs) within neuronal components of this region. Positive immunostaining was localised to nerves associated with anterior nerve ring, metacorpal bulb, subventral and dorsal gland ampullae. The data support a significant role for FaRPs in the control of feeding activities in PCN.

MICRO-GENOMIC APPROACHES TO THE DEVELOPMENT OF NEMATODE CONTROL. **Huang, X., Y. Han, H. Jia, L. Xing, R. Zhen, and S. Chaudhuri.** BASF Plant Science, L. L. C., Research Triangle Park, NC 27523.

The soybean cyst nematode (SCN), *Heterodera glycines,* penetrates the soybean root and induces cells near the vascular tissue to form a highly specialized feeding site termed syncytium. The syncytium is very unique and dramatically different from its neighboring tissues. It serves as a nutrient sink that supports the nematode life cycle. In order to discover targets, it is essential to understand the mechanism of syncytium formation and development at the molecular level. However, it is technically impossible to isolate the syncytium from its neighboring normal root cells using standard techniques. We have attempted the recently developed Laser Capture Microdissection (LCM) technology that entails the isolation of pure population of cells from heterogeneous tissue specimens, coupled with downstream RNA amplification and microarray expression analysis to the study of gene expression in syncytia. Comparison of gene expression between syncyta and its neighboring tissues and the uninfected roots has led to the identification of around 300 genes that are uniquely regulated in syncytia. Further experiments and bioinformatics analysis has been conducted to identify candidates for gene expression analysis. This study represents, to the best of our knowledge, the first ever such comprehensive analysis of gene expression in syncytia or any other specific cell types in plants. Our successful implementation of LCM and downstream expression analysis in the study of syncytia has greatly increased the chances of discovering genes that otherwise could have gone un-detected in heterogeneous tissue specimens.

VALIDATION PROTOCOL TO DETERMINE THE SPECIFICITY AND SENSITIVITY OF MOLECULAR DIAGNOSTIC FOR LONGIDORID NEMATODES. **Hübschen, J.,[1] L. Kling,[1] U. Ipach,[1] V. Zinkernagel,[2] N. Bosselut,[3] D. Esmenjaud,[3] D. J. F. Brown,[4] and R. Neilson.[5]** [1]Dienstleistungszentrum Ländlicher Raum Rheinpfalz, Breitenweg 71, 67435 Neustadt/Weinstrasse, Germany; [2] Technische Universität München, Lehrstuhl für Phytopathologie, Am Hochanger 2, 85350 Freising-Weihenstephan, Germany; [3] Institut National de la Recherche Agronomique, BP 2078, 06606, Antibes, France; [4] Central Laboratory of General Ecology, 2 Gagarin Street, 1113 Sofia, Bulgaria; [5] Scottish Crop Research Institute, Dundee, DD2 5DA, Scotland, UK.

Few of the published molecular diagnostics for ecto- and endoparasitic nematodes have undergone rigorous evaluation to determine their specificity and sensitivity. Typically, evaluations have been limited to testing designed primers with target species (usually ten or less) under study without consideration of closely related species (taxonomic or functional) from the same habitat. Furthermore, possible cross-reactions with species from the general nematode community have rarely been considered. Evaluation of the sensitivity of diagnostic primers has also been cursory with artificial laboratory tests comparing target and non-target nematode in unrealistic ratios (e.g. 1:1) especially when target nematodes typically occur in the field, in ratios of 1:>200 non-target nematodes. Here we detail a protocol that determines the specificity of diagnostic primers for longidorid nematode species that are economically important to the European viticulture industry. An experimental dilution series of extracted DNA rigorously demonstrated that DNA from an equivalent single target specimen could be detected amongst 1000 equivalent non-target specimens from the same genus, as well as total DNA extracted from a soil nematode community. Diagnostic primers were assessed further by using serial mixtures of actual nematodes rather than extracted DNA to simulate a field scenario. Using this method, a single target nematode could be detected amongst 200 non-target nematodes.

SECRETS IN SECRETIONS: GETTING TO THE ROOTS OF NEMATODE PARASITISM OF PLANTS. **Hussey, R. S.** Department of Plant Pathology, University of Georgia, Athens, GA 30602-7274.

Stylet secretions encoded by parasitism genes expressed in the esophageal gland cells mediate nematode parasitism of plants. More than 70 parasitism genes have been cloned from *Heterodera glycines* and over 50 from *Meloidogyne incognita*, with over 70% of the parasitism genes identified having no significant similarity to any reported genes. Furthermore, few similarities exist among the parasitism genes between these two nematode species, suggesting that the molecular tools they use to parasitize plants might differ considerably. In addition to parasitism genes encoding secretory cell-wall degrading enzymes, these nematodes appear to possess a wide diversity of parasitism genes encoding secretory proteins that enable them to parasitize and modify root cells into feeding cells. These include parasitism proteins mimicking plant peptide signals, novel ubiquitin extension proteins, and proteins that could influence protein degradation, cell-cycle modulation, and cellular metabolism, in addition to an array of parasitism proteins unique to plant-parasitic nematodes. Identification of nematode parasitism genes represents a major step towards dissecting the molecular interactions in nematode parasitism of plants. These important discoveries are not only providing fundamental knowledge of nematode parasitism genes and their functions, but will provide multiple targets to interfere with in the parasitic process as potential control mechanisms to limit nematode-induced crop losses.

SUPPRESSION SUBTRACTIVE HYBRIDIZATION IDENTIFIED A GENE REQUIRED FOR *Mi-1.2*-MEDIATED NEMATODE RESISTANCE AND *Mi-DS4*-MEDIATED CELL DEATH. **Hwang, C. F., and V. M. Williamson.** Department of Nematology, University of California, Davis, CA 95616.

The tomato gene *Mi-1.2* belongs to the nucleotide-binding/leucine-rich repeat (NB-LRR) family of plant resistance genes and confers resistance against several root-knot nematode species. To study *Mi-1.2* function, we have produced a recombinant form of *Mi-1*, *Mi-DS4*, that signals a nematode-independent cell death response after about three days when transiently expressed in *Nicotiana benthamiana* leaves. The strong correlation of the mutations that produce a loss-of-function in the root nematode resistance assay with those causing loss of *Mi-DS4*-induced necrosis suggests that the pathways leading to nematode resistance and leaf necrosis are related. We have used suppression subtractive hybridization to identify 84 unique candidate cDNAs whose mRNAs may be more abundantly expressed 48 hours after transient expression of *Mi-DS4*. One of these encodes ACC oxidase, which catalyses the conversion of ACC to ethylene. Silencing of *ACC oxidase* by potato virus X-induced gene silencing compromised the *Mi-DS4*-induced cell death. In addition, cobalt chloride, an ACC oxidase inhibitor, blocks *Mi-DS4*-mediated cell death in *N. benthamiana* leaves and abolishes nematode resistance in tomato roots. These results suggest that ACC oxidase is required for both *Mi-DS4*-mediated cell death and for *Mi-1.2*-mediated nematode resistance. Thus, the availability of genes that are differentially increased in expression preceding *Mi-DS4*-mediated cell death provides an excellent source of candidate genes for signaling components leading to *Mi-1.2*-mediated nematode resistance.

COMPARATIVE MERMITHID MITOCHONDRIAL GENOMICS. **Hyman, B. C.,[1,2] E. G. Platzer,[1,2] S. Tang,[3] and Z. Wu.[4]** [1]Departments of Biology and [2]Nematology; Interdepartmental Graduate Programs in [3]Genetics and [4]Cell, Molecular and Developmental Biology, University of California, Riverside, CA 92521.

The mitochondrial genome of the obligate mosquito parasite *Romanomermis culicivorax* features a unique architecture characterized by lengthy 3.0 kilobase repeating units present in both tandem head-to-tail arrays and as unlinked inverted copies. To deduce the molecular events sponsoring the generation of this novel mitochondrial DNA organization, complete nucleotide sequence and mitochondrial gene orders were determined for the mitochondrial genomes of *R. culicivorax*, the basal congener *R. iyengari*, as well as another representative of the order Mermithida, *Thaumamermis cosgrovei*. Partial gene orders for mitochondrial genomes from the mermithids *R. nielseni, Mermis nigrescens* and an *Ovomermis* sp. have also been deduced. Lengthy repeating units, often containing mitochondrial genes, appear to be a frequent architectural feature of enoplean mitochondrial genomes, but are not observed within the chromodorean mitochondrial DNAs sequenced to date. Conserved syntenic relationships among mitochondrial gene orders available for the mermithid nematodes, another enoplid (*Trichinella spiralis*) and more distant representatives of the class Chromadorea enable prediction of ancestral nematode mitochondrial gene clusters and possible events leading to the unusual *R. culicivorax* mitochondrial genome organization.

CHALLENGES TO MANAGEMENT OF ROOT-KNOT NEMATODE DAMAGE TO POTATO CAUSED BY MIXED POPULATIONS. **Ingham, R. E.,[1] H. Mojtahedi,[2] and C. R. Brown.[2]** [1]Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331; [2]USDA-ARS, Prosser, WA 99350.

Root-knot nematodes are serious pathogens of potato (*Solanum tuberosum*) in the western United States, causing quality defects that result in reduced crop value or crop rejection. *Meloidogyne chitwoodi* is generally considered to be the most damaging species and has been reported from nearly all states west of the Rocky Mountains. *Meloidogyne hapla* also occurs in many western states and may be found alone or with *M. chitwoodi*. Southwestern states may also have populations of *M. incognita, M. javanica or M. arenaria*, but these areas account for less than 8% of potato production in western states. Two races are recognized for *M. chitwoodi* and can only be distinguished by differential host test, race 1 reproducing on 'Chantenay' carrot and not on 'Thor' alfalfa and race 2, which reproduces on alfalfa but not on carrot. A third isolate, originally identified as race 3, is the only known *M. chitwoodi* to establish on the clonal selection P1275187.10 of *Solanum bulbocastanum*, breaking resistance conditioned by *RMc1(bib)* gene. Race 3 has since been considered as a virulent pathotype of race 2. Both races appear equally damaging to tuber quality, with the potential to cause economic damage at or below the detection limit of 1/250 cm3 soil. Populations of *M. chitwoodi* containing both races may also occur. The inability to easily identify and distinguish races introduces serious restrictions to developing cultural management strategies. For example, crop rotation to alfalfa would suppress race 1 but not race 2. In addition, potato-breeding programs have found it more difficult to attain resistance to race 2 than to race 1.

USE OF REAL-TIME PCR TO DETERMINE RELATIVE QUANTITIES OF FUNGAL AND PLANT DNA WITH APPLICATIONS FOR STUDYING NEMATODE-FUNGAL INTERACTIONS. **Jackson, T. A., X. Gao, K. N. Lambert, and T. L. Niblack.** Department of Crop Sciences, University of Illinois, Urbana, IL 61801.

Estimates of fungal colonization are useful to determine the effects of nematode parasitism on fungal infection of plants. Traditional methods employed to evaluate fungal colonization involve serial dilution platings on selective media, which is labor-intensive and time-consuming. Relative real-time quantitative PCR (QPCR) has been recently developed as a rapid alternative method for quantifying fungal colonization. Relative quantification is favored under circumstances where knowledge about relative changes in gene expression is more meaningful than exact copy numbers of genes. Research was undertaken to compare a traditional plating method and relative QPCR to estimate *Fusarium solani* f. sp. *glycines* (Fsg), causal agent of soybean sudden death syndrome, in soybean roots. Dried root samples were suspended in water, serially diluted, and plated on a semi-selective medium to determine the number of colony-forming units (cfu) of Fsg per gram of root. Total DNA was extracted from 152 dried root samples and subjected to QPCR to determine relative quantities (RQ) of Fsg DNA to soybean DNA. QPCR detected and amplified Fsg DNA in 85% of the samples, whereas Fsg was detected in only 28% of samples with the traditional plating method. The RQ of Fsg DNA to soybean DNA were not correlated ($p = 0.8769$) to the number of Fsg cfu determined by the traditional plating method. Samples were processed and evaluated at least 14 days faster for QPCR. Preliminary data suggests that QPCR is a good alternative method for evaluating Fsg colonization of soybean roots as affected by soybean cyst nematode population densities.

IMPACT OF SUBSTRATES AND PULSING AGENTS ON REPRODUCTION OF BACTERIAL-FEEDING NEMATODES. **Jost, M.-S., J. Biernbaum, G. Bird, C. Bates, S. Hollosy, M. Quintanilla, and J. Smith.** Department of Entomology, Michigan State University, East Lansing, MI 48824.

As a student in a MSU nematology course in 2002, the senior author made numerous observations on nematodes associated with composts, leading to studies of nematode reproduction in compost (substrate) amended with an alfalfa-based Bradfield Inc. organic fertilizer (pulsing agent). A vessel was developed for controlling and monitoring temperature, CO_2/O_2 gas exchange rate, substrate moisture content, electrical conductivity and pH. Nematode population densities increased from 250 to 2,866/100 cm3 after 7 days, with substrate temperatures increasing to as high as 46 C in the vessel, but increased to 13,025/100 cm3 with maintained cooling of the substrate at 22 C. In vessels without the pulsing agent there

were no increases in nematode population densities and the substrate remained a constant 22 C. In the spring of 2004, six students in a MSU nematology course conducted three experiments to evaluate the impacts of various substrates and pulsing agents on population density increase of bacterivores and oligocheates. Increases in system temperature and nematode reproduction were substrate dependent. Organic pulsing agents varied significantly in their ability to influence system temperature and population densities of nematodes. There appears to be an optimal ratio between substrate, pulsing agent and system moisture potential for maximizing the reproduction of bacterial-feeding nematodes. Oligocheates were only recovered when system temperatures were not elevated.

UPTAKE OF PLASTID COMPONENTS BY MIGRATORY ENDOPARASITIC NEMATODES. **Khaithong, T.,[1] B. S. Sipes,[1] and A. R. Kuehnle.[2]** [1]Department of Plant and Environmental Protection Sciences; [2]Department of Tropical Plant and Soil Sciences; University of Hawaii at Manoa, 3190 Maile Way, Honolulu, HI 96822.

Plant plastid transformation offers critical advantages over nuclear transformation and is a promising technology for the development of nematode resistant plants. However, this strategy requires the uptake of resistant proteins by nematodes, which was the objective of this research. Conclusive evidence of uptake of plastid components by nematodes is needed. *Pratylenchus penetrans* was fed on calli of tobacco transgenic for GFP protein that is transported to the thylakoid lumen of the chloroplast. After feeding, the nematodes were observed under a confocal laser scanning microscope and compare to nematodes fed on alfalfa callus. The intestine of nematodes that fed on GFP transformed tobacco showed more intense green fluorescent than the ones that fed on alfalfa root callus. Observation was limited by the green auto-fluorescent of the nematodes however. *Radopholus similis* and *P. penetrans* were cultured on carrot disks in light. Nematodes feeding on green carrot calli were directly picked into 20 µl of 1% glycine with protease inhibitor in a 0.6 ml microcentrifuge tube. Nematodes were macerated and centrifuged. The supernatant was collected and a Western blot analysis was performed to detect the small subunit Rubisco protein, a protein prevalent in the chloroplast. Nematodes fed on orange carrot tissue and alfalfa callus were used for comparison. Dot blot assay showed positive result in the protein samples from *P. penetrans* fed on orange and green carrot callus, adding further evidence that the nematodes consume plastid components.

MANAGEMENT OF ROOT-KNOT NEMATODE BY BIOGAS SLURRY IN POTTED SOIL. **Khan, Zakaullah, and So Deuk Park.** Seongju Fruit-Vegetable Experiment Station, Gyongbuk Province, Republic of Korea.

Pot experiments were conducted to evaluate the effect of biogas slurry as an amendment for the management of root-knot nematodes, *Meloidogyne arenaria* in tomato. Amending the potting mixture with biogas slurry at rates of 10, 20, 30, 40 and 50% v/v, reduced the root galling and final nematode population of *M. arenaria* and increased tomato plant growth ($P < 0.001$), compared with the non-amended control. In the non-amended potting mixture *M. arenaria* caused root galling on more than 80% of tomato root system and the final nematode population increased 14.6 folds after 2 months of inoculation, compared with initial nematode density. Increasing the rate of that amendment exponentially reduced the root galling caused by *M. arenaria* (17.4–58.5%) and the final nematode population (40.4–95.3%) and increased plant growth 15.5–72.5%)compared to non-amended control. However, suppression of tomato root galling and final population of *M. arenaria* was not influenced by increasing amendment rate from 30 to 50% ($P < 0.001$). Our results demonstrated that amending potting mixture with biogas slurry suppressed root-knot disease of tomato plant caused by *M. arenaria* and enhance plant growth. Disease suppression was expressed both by reducing the amount of root galling and the amount of secondary nematode inoculum.

EFFICACY OF *PAECILOMYCES LILACINUS* STRAIN 251 FOR BIOLOGICAL CONTROL OF THE BURROWING NEMATODE *RADOPHOLUS SIMILIS.* **Kiewnick, S., A. Mendoza, and R. A. Sikora.** University of Bonn, Department of Soil Ecosystem Phytopathology and Nematology, Nussallee 9, D-53115 Bonn, Germany.

Radopholus similis causes serious economic losses in Musa spp. worldwide. *Paecilomyces lilacinus* strain 251 is currently registered in the Philippines as BIOACT®WG for control of plant parasitic nematodes on banana. Due to new and advanced fermentation and formulation technologies is was necessary to re-evaluate the potential of *P. lilacinus* strain 251 for the control of *R. similis*. The effects of time of application and dose were assessed in pot experiments using Grande Naine banana plantlets. Conidia of *P. lilacinus*, formulated as water dispersible granules, were applied to soil inoculated with *R. similis* 6 days before, at, and 6 days after transplanting banana plantlets. Fourteen days after transplanting, plantlets were assessed for nematode numbers and biomass production. A significant correlation between the rate applied (0; 0,2 and 0,4g product/plant) and the number of nematodes per root was found only when *P. lilacinus* was applied as a pre-planting soil treatment. Application at or after transplanting resulted in insufficient reduction of *R. similis*. Additionally, dose response experiments with 7 rates applied as pre-planting treatment resulted in a highly significant correlation between the amount of product applied and the number of nematodes per root ($r^2 = 0{,}850$). Furthermore, the combination of a pre-planting application, plantlet drench 24 hours before transplanting and at-planting treatment resulted in a significantly better reduction in the number of nematodes per root (82%) compared to single applications (36 and 65%). *P. lilacinus*

strain 251, although primarily known as a facultative egg pathogen of sedentary nematodes, demonstrated a sufficient efficacy as commercially formulated biological nematicide for control of *R. similis*.

BIOLOGICAL CONTROL OF THE SUGAR BEET CYST NEMATODE *HETERODERA SCHACHTII* WITH *PAECILOMYCES LILACINUS* STRAIN 251. **Kiewnick, S., A. Schmitz, and R. A. Sikora.** University of Bonn, Department of Soil Ecosystem Phytopathology and Nematology, Nussallee 9, D-53115 Bonn, Germany.

Biological nematicides can play an important role in the integrated control of plant parasitic nematodes. However, the number of commercialised products is still low due to restrictions in the cost effective production and formulation of biocontrol agents. The egg pathogenic fungus *Paecilomyces lilacinus* strain 251, is a unique strain showing activity against a range of plant parasitic nematodes. *P. lilacinus* strain 251 has already demonstrated activity against root-knot and potato cyst nematodes in the past and the potential of this biocontrol agent for control of the sugar beet cyst nematode was evaluated. In pot experiments, *Heterodera schachtii* cysts were exposed to different concentrations of commercially formulated conidia of *P.lilacinus* strain 251 ranging from LOG 4 to LOG 7 conidia/g soil. Evaluation of the percentage infected eggs after 14 days incubation demonstrated a clear dose response. At 25 °C, the highest concentration resulted in more than 50% infected eggs. Furthermore, untreated cysts and cysts exposed to soil treated with LOG 6 and LOG 7 conidia/g soil, respectively for 14 days were used to inoculate sugar beet seedlings. Fourteen days after inoculation, the number of nematodes derived from the sugar beet root system was reduced by 50 and 75%, respectively. This indicated that the level of infected eggs correlated well with a reduced penetration of the host plant. Additionally, root weight and root length of sugar beets were increased at the high rate fo fungus in the soil. The egg pathogenic fungus *P. lilacinus* strain 251 showed potential to be integrated into existing programs for the control of the sugar beet cyst nematode *H. schachtii*.

PHYLOGENY OF RHABDITID NEMATODES: MOLECULAR AND MORPHOLOGICAL EVIDENCE. **Kiontke, K.,[1] W. Sudhaus,[2] and D. H. A. Fitch.[1]** [1]Department of Biology, New York University, New York, NY 10003; [2]Institut für Zoologie, Freie Univeristät Berlin, 14195 Berlin, Germany.

To reconstruct a phylogenetic tree of "Rhabditidae" with molecular data we are sequencing small and large subunit ribosomal RNA genes (SSU rDNA and LSU rDNA), and part of the gene for the largest subunit of RNA polymerase II (RNAP2) for representatives of all genera within "Rhabditidae", representatives of Diplogastridae and Strongylida and some cephalobids and panagrolaims as representatives of the outgroup. Preliminary analyses show high support for the monophyly of "Rhabditidae" plus Strongylida and Diplogastridae. Some monophyletic clades within "Rhabditidae" are also well established (e.g. *Caenorhabditis; Oscheius;* a clade consisting of *Mesorhabditis, Pelodera, Teratorhabditis* and *Parasitorhabditis;* Diplogastrina). Within most of these clades, relationships are fairly well resolved on the species level, although for Diplogastrina, our molecular data are insufficient to resolve their phylogeny in detail. We are also collecting morphological data for all species chosen for the molecular phylogenetic study. Our data matrix includes more than 100 different morphological characters plus some ecological, developmental, behavioral and life-history characters. In addition, we are collecting data on intron distribution in RNAP2. We are comparing phylogenetic trees resulting from analyses of the molecular and morphological datasets. For *Caenorhabditis*, the branching pattern obtained with molecular data is largely congruent with the phylogenetic tree reconstruced previously using morphological characters. The clade consisting of *Mesorhabditis, Parasitorhabditis* and *Teratorhabditis* is also supported by morphological apomorphies, i.e. a posterior vulva and loss of the anterior ovary.

IMPACT OF N-VIRO SOIL ON *HETERODERA GLYCINES* WITH RESISTANT AND SUSCEPTIBLE CULTIVARS. **Koenning, S. R.** Department of Plant Pathology, North Carolina State University, Raleigh, NC 27695-7616.

Field and microplot experiments with N-Viro Soil (N-Viro International, Englewood, Ohio USA) were conducted in North Carolina in from 2002-2004 to evaluate the effects of NVIRO SOIL on Heterodera glycines (SCN) with SCN resistant and susceptible cultivars. Both experiments were performed in a Fuquay sand. The field experiment utilized a naturally infested field with SCN race 5, whereas SCN race 4 was used in the microplot trial. Selected plots had 0, 5, 10, or 15 tons/Acre of NVIRO SOIL broadcast on the soil surface. Both experiments used a 2 × 4 factorial with resistant and susceptible cultivars (SCN resistant cv.s Delsoy 5710 in microplots, and Fowler in the field trial, and susceptible Hutcheson) and four rates of NVIRO SOIL (0, 11, 22, and 33 mt/ha) arranged as a RCBD. Resistant cultivars yielded more and had lower ($P < T\ 0.01$) harvest population densities of SCN compared to susceptible Hutcheson. Final population densities of SCN tended to increase linearly with application of N-Viro Soil in both microplot and field experiments ($P < T\ 0.10$).

EFFECTS OF PRE- AND POST-PLANT APPLICATIONS OF *STEINERNEMA RIOBRAVE* ON ROOT-KNOT NEMATODE POPULATIONS AND GALLING OF TOMATO. **Kokalis-Burelle, N.,[1] and E. E. Lewis.[2]** [1]USDA, ARS, U.S. Horticultural Research Lab, Fort Pierce, FL 34945; [2]Virginia Tech University, Blacksburgh, VA 24061-0319.

Several rates of the entomopathogenic nematode (EPN) *Steinernema riobrave* applied pre- and post-plant were evaluated for control of *Meloidogyne incognita* on tomato in Florida microplots. Experiments were performed in spring and repeated

in fall of 2001. Treatments were four pre-plant rates of *S. riobrave*, untreated, 0.5, 1.0, and 2.0×10^9 infective juveniles (IJ)/a, combined with one post-plant application rate of 1×10^9 (IJ)/a. Root-knot nematode (RKN) populations were assessed pre-plant/pre-treatment, post-treatment, 7 days after planting (DAP), 14 DAP, after post-plant treatment, and at end of season. In the spring, with cool soil temperatures in the early season, application of additional EPN after planting improved plant growth and root condition but did not significantly reduce galling. Area under the development curve for RKN populations showed low rates of EPN applied early in the spring increased numbers of RKN isolated from soil while higher rates reduced them. The combination of high pre-plant rates and post-plant application of EPN reduced numbers of RKN juveniles isolated from soil over the growing season to the greatest degree. In the fall, soil temperatures were high early in the season and the post-plant application of EPN did not enhance plant growth, root condition or reduce galling. However, higher pre-plant rates of EPN had lower amounts of galling while more RKN juveniles were isolated from soil over the season. The fact that populations remained high but the juveniles did not infect the roots indicates that EPN may interfere with RKN mobility or host location. Also, soil temperature at the time of EPN application can greatly affect results.

HOST- RANGE STUDIES FOR *MELOIDOGYNE FLORIDENSIS*. **Kokalis-Burelle, N.,[1] and A. P. Nyczepir.[2]** [1]USDA, ARS, U.S. Horticultural Research Laboratory, Fort Pierce, FL 34945; [2]USDA, ARS, SE Fruit & Tree Nut Research Laboratory, Byron, GA 31008.

Meloidogyne floridensis has recently (2004) been characterized as a new root-knot nematode species although it was initially identified in 1982 as *M. incognita* race 3. This nematode was first detected in 1966 in Gainesville, Florida, on a site cleared of native timber and used for peach rootstock trials. The nematode was reported to reproduce on root-knot nematode resistant Nemaguard and Okinawa peach rootstocks. In the 1970's, this nematode was detected in additional surrounding sites previously planted to soybeans, corn, lupine and red clover. Host range studies were performed to determine the reproductive potential (RP) of *M. floridensis* on several vegetable, ornamental, and herb crops important to Florida, where this nematode has been isolated with increasing frequency. Crops tested were tomato, bell pepper, cucumber, eggplant, squash, collards, strawberry, impatiens, marigold, verbena, snapdragon, begonia, parsley, sage, basil, and dill. Plants were potted in a nematode-free sand:peat moss mix, inoculated with 5,000 *M. floridensis* eggs/1,000 cm^3 soil, and grown in the greenhouse for 30 days. Eggs were extracted from roots and RP was calculated using Pf/Pi ratios. The highest levels of *M. floridensis* reproduction were observed on verbena (12.6), eggplant (8.1), squash (7.6), and basil (6.8), followed by intermediate reproduction levels for impatiens (3.3), tomato (1.9), snapdragon (1.6), and dill (1.2). Sage, marigold, cucumber, begonia, parsley, collards, strawberry and pepper had RP values <1.0 and were considered non-hosts for *M. floridensis*. More extensive host range tests including weeds common to Florida are currently underway.

THE COMPARATIVE EFFECTS OF *PRATYLENCHUS PENETRANS* INFECTION AND *MALADERA CASTANEA* FEEDING ON STRAWBERRY BLACK ROOT ROT. **LaMondia, J. A., and R. S. Cowles.** The Connecticut Agricultural Experiment Station Valley Laboratory, Windsor, CT 06095.

The interaction of lesion nematodes, root damage caused by feeding of the Asiatic garden beetle larva (*Maladera castanea*), and the black root rot fungus (*Rhizoctonia fragariae*) on strawberry root disease was determined in three greenhouse studies. Averaged over all experiments after twelve weeks, root weight was reduced by 13% by *R. fragariae* and by 20% by *M. castanea*. Inoculation with *P. penetrans* did not affect root or shoot weights. The percent of the root system affected by root rot was increased by inoculation with either *R. fragariae* (35% more disease) or *P. penetrans* (50% more disease), but was unaffected by *M. castanea*. *Rhizoctonia fragariae* was isolated from 9.2% of the root segments from *R. fragariae*-non-inoculated plants, but was increased 3.6-fold following inoculation with *R. fragariae* on rye seeds. The presence of *P. penetrans* in strawberry roots also increased the proportion of the root system infected with *R. fragariae* and the severity of root rot. The type of injury to root systems was important in determining whether roots were invaded by *R. fragariae* and increased the severity of black root rot. For example, weakened or dying cells resulting from the direct or indirect effects of *P. penetrans* movement and feeding were more susceptible to *R. fragariae* and had more infection and cortical root rot, whereas the traumatic cutting action by chewing insects did not increase root infection or root disease. While both insects and diseases need to be managed to extend the productive life of perennial strawberry plantings, in these experiments, only *P. penetrans* and *R. fragariae* interacted to increase the severity of cortical root rot.

EFFECT OF VISIBLE, A SOIL ADDITIVE, ON THE GROWTH OF COTTON IN A RENIFORM NEMATODE INFESTED FIELD. **Lawrence, G. W.,[1] and K. S. Lawrence.[1,2]** Department of Entomology and Plant Pathology, Mississippi State University, Mississippi State, MS 39762; [2] Department of Entomology and Plant Pathology, Auburn University, Auburn, AL 36849.

Visible, a soil penetrant and enhancer, was examined to determine if enhanced root and plant growth would benefit cotton production when used in the presence of the reniform nematode. The test was conducted in an established cotton production location and naturally infested with the reniform nematode (*Rotylenchulus reniformis*). Visible (0.59, 0.85 and

1.20kg a.i./ha) was compared with aldicarb (0.85kg a.i./ha) and both were applied at planting with a planter equipped with granular chemical applicators. Stoneville 4892 BR cotton plants were taller, had more open bolls and higher cotton weights in the Visible and aldicarb treatments compared with the control. The first open boll was on fruiting branch 5.7, 6.1 and 7.4 for aldicarb, Visible and the control, respectively. Seed cotton yields in the visible treatments were increased an average of 373.88 kg /ha over the control. Reniform nematode numbers averaged across the season were 408, 632 and 931reniform nematodes /250 cm^3 soil in aldicarb, Visible and the control.

ACCELERATED BIODEGRADATION OF AlLDICARB AND ITS METABOLITES IN COTTON FIELD SOILS **Lawrence, K. S.,[1] F. Yucheng,[2] G. W. Lawrence,[3] C. H. Burmester,[2] and S. H. Norwood.[2]** [1]Department of Entomology and Plant Pathology, Auburn University, Auburn, AL 36849 ; [2]Agronomy and Soil Science, Auburn University, Auburn, AL 36849; [3]Department of Entomology and Plant Pathology, Mississippi State University, MS 39762.

The degradation of aldicarb, aldicarb sulfoxide, and aldicarb sulfone was evaluated in cotton field soils previously exposed to aldicarb. Two soils were selected for the first test, one where aldicarb was effective (CL) and the second where aldicarb had lost its efficacy (LM). In the second test, the efficacious CL soil was compared to the LM and MS soils where aldicarb had lost its efficacy. These soils were autoclaved or natural (non-autoclaved) and treated with aldicarb 0.59 kg a.i./ha or not treated with aldicarb. The degradation of aldicarb to aldicarb sulfoxide and than to aldicarb sulfone was determined using HPLC in both tests. In test one, total degradation of aldicarb and metabolites occurred within 12 days in the LM soil. Aldicarb sulfoxide and aldicarb sulfone were both present in the CL soil at the conclusion of the test at 42 days after aldicarb application. Aldicarb degradation was enhanced in the LM and MS natural soils compared to the same soils when autoclaved. Degradation was not enhanced in the CL natural soil as compared to the CL autoclaved soil. The loss of aldicarb efficacy was due to a more rapid degradation of the aldicarb sulfoxide metabolite and appears to be biologically mediated.

THE ENDOSYMBIONT BACTERIA OF *XIPHINEMA AMERICANUM*-GROUP NEMATODES. **Lazarova, S. S.,[1] D. J. F. Brown,[1] G. Malloch,[2] C. M. G. Oliveira,[2, 3] B. Fenton,[2] and R. Neilson.[2]** [1]Central Laboratory of General Ecology, 2 Gagarin Street, 1113 Sofia, Bulgaria; [2] Scottish Crop Research Institute, Dundee, DD2 5DA, Scotland, UK; [3] Instituto Biológico, Campinas, P.O. 70, 13901-970, SP, Brazil.

A characteristic of the *Xiphinema americanum*-group of nematodes is the presence of obligate intracellular endosymbiont bacteria that have previously been identified as belonging to the division *Verrucomicrobia*. The associations with cytoplasmically inherited microorganisms are of great importance to the ecology and reproductive biology of many invertebrates including nematodes. *Xiphinema americanum*-group populations were collected from a number of geographically disparate locations and we characterized the molecular diversity of endosymbiont bacteria and determined the specificity of the endosymbiont-nematode association. Nematode and bacterial DNA were extracted simultaneously using a lysis method and the 16S (bacteria) and 18S (nematode) ribosomal genes and the mitochondrial gene CO I were sequenced. Approximately 50% of the bacterial sequences obtained from the 34 nematode populations studied were identical or shared >97% 16S rDNA homology to one of the previously described sequences from *X. americanum*, *X. brevicollum* and *X. rivesi*. Furthermore, potentially eight new bacterial strain groups were distinguished based on sequence dissimilarity of >3%. Phylogenetic trees of all three genes were compared to determine the likely mode of transmission of the bacteria to the nematode hosts. Preliminary analysis suggests the most probable mode of transmission is vertical (i.e. maternal).

EFFECT OF APPLICATION RATE AND TIMING OF CASTOR BEAN CAKE FOR THE CONTROL OF ROOT-KNOT NEMATODE. **Lee, Jae-Kook,[1] Dong-Geun Kim,[2] and Dong-Ro Choi.** [1]Entomology Division, National Institute of Agricultural Science and Technology, R.D.A., Suwon, Korea; [2]Gyeongbuk Agricultural Technology Adminstration, Daegue, Korea.

For the control of root-knot nematode, *Meloidogyne arenaria*, pot test was conducted. Castor bean cake was mixed with nematode-infested soil for 1, 3, 5, 7, and 11 weeks before planting at the rate of 600kg, 1200kg, 2,400kg/10a, respectively. The amount of application was more important than application timing to reduce nematode reproduction. The number of soil bacteria and saprophytic nematodes were positively correlated with the amount of application, and the population density peaked after five weeks of treatment then declined. When castor bean cake was treated 11 weeks before planting at the rate of 2,400kg/10a, it inhibited nematode reproduction as much as 98% compared to the control.

CONTROL OF ORIENTAL FRUIT MOTH USING ENTOMOPATHOGENIC NEMATODES. **Leger. C.,[1] E. Riga,[2] and L. Lacey.[3]** [1]Department of Plant Pathology, Washington State University, Pullman, WA 99163; [2]IAREC and Department of Plant Pathology, Washington State University, Prosser, WA 99350; [3]USDA-ARS Yakima Agricultural Research Laboratory, Wapato, WA 98951.

The objective of this research was to evaluate the use of entomopathogenic nematodes (EPN) to control oriental fruit

moth (Grapolita molesta), a serious pest of stone fruit. EPN are characterized by vector a pathogenic bacteria which kills the host within 24–48 h, showing considerable potential in biological control of insect pests. Early in season, larvae bore into the tips of tender twigs while later the larvae bore into the fruit causing severe losses. Since control management have been based in the application of insecticides the population of OFM has developed resistance, which create the necessity of new alternative pest management strategies. The susceptibility of the OFM diapausing larvae to four species of EPN was evaluated in laboratory experiments using cocooned larvae inside cardboard strips. The EPN species tested were Steinernema carpocapsae, Steinernema feltiae, Heterorhabditis marelatus, and Heterorhabditis bacteriophora. Petri dishes contained 20 larvae of G. molesta were applied with concentrations of 5, 10 and 20 infective juveniles/cm2, and incubated for 48 h at 25 °C with five replications for each nematode species. All EPN species were effective in controlling OFM larvae. The preliminary results show that the most virulent EPN species is S. feltiae with high range of mortality 43–86%, and S. carpocapsae with 41−79 % at a concentration of 5 and 20 IJ's/cm2 respectively in both cases, followed by H. marelatus with 24−70 %, and H. bacteriophora with 5−32 % mortality. Further work is in the process to determinate the LC50 and LC95.

SUSCEPTIBILITY OF SEVERAL PERENNIAL ORNAMENTALS TO FOUR SPECIES OF ROOT-KNOT NEMATODES. **Levin, R.,[1] J. Brito,[2] W. T. Crow,[1] and R. K. Schoellhorn.**[1] [1]University of Florida, Gainesville, FL 32611; [2]Division of Plant Industry, Gainesville, FL 32608.

The susceptibility of *Pittosporum tobira* 'Variegata', *Liriope muscari* 'Evergreen Giant', *Salvia leucantha*, and *Odontonema cuspidatum* to *Meloidogyne incognita* Race 2, *M. javanica*, *M. arenaria* Race 1, and *M. mayaguensis* was evaluated in separate experiments for each plant species. Each experiment consisted of the four *Meloidogyne* spp., which were inoculated on 30 plants that were arranged in a randomized complete block design with six replications. Each plant was inoculated with 5000 eggs and juveniles of a respective *Meloidogyne* spp., or remained uninoculated. The plants were maintained for at least 60 days in a temperature and light-controlled growth room for the duration of each experiment, watered daily, and fertilized weekly. Plants were then harvested by replication. Root galling index, number of *Meloidogyne* eggs and juveniles, shoot dry weights, and root fresh weights were compared among nematode species. Results suggest that *L. muscari* 'Evergreen Giant' is a good host to all of the *Meloidogyne* spp. isolates tested except for *M. arenaria*. *Salvia leucantha* and *P. tobira* 'Variegata' were good and poor hosts to the *Meloidogyne* spp. isolates tested, respectively. No reproduction was observed for any of the *Meloidogyne* spp. isolates tested on *O. cuspidatum.*

NEMATICIADAL BT CRYSTAL PROTEINS TARGETING PLANTS ENDOPARASITIC NEMATODES. **Li, Xiang-Qian, Jun-Zhi Wei, Raffi Aroian.** Department of Biological Sciences, University of California- San Diego, La Jolla, CA 92093.

Plant-parasitic nematodes (PPNs) cause significant economic losses worldwide. The current control strategies of PPNs include crop rotation, resistant varieties, chemical nematicides, and Integrated Pest Management. Under the Montreal Protocol, the main chemical nematicide, methyl bromide, is supposed to be phased out in developed countries in 2005, although the US government has asked for an extension. Transgenic plants expressing the environment-friendly and vertebrate non-toxic insecticidal crystal proteins of *Bacillus thuringiensis* are one of the modern breakthroughs in crop sciences. It has resulted in significant reductions in toxic chemical pesticide use and in significant gains in crop yields. Our laboratory has demonstrated that *E. coli* expressing Cry proteins present in the Cry5 and Cry6 subclades are toxic to phylogenetically diverse free-living nematodes. Among these tested nematodes, *Acrobeloides* sp. belongs to the subclade of free-living nematodes closely related to the Tylenchida. These data suggest for the first time that Bt Cry proteins might have utility in controlling parasitic nematodes. Since to our knowledge it was not being tested but felt that it should be, we thus set out to test the hypothesis that Cry proteins expressed in transgenic plants might provide protection against plant-parasitic nematodes. To begin with, we are testing this hypothesis with Cry6A protein. In order to increase the expression level in transgenic plants, we synthesized "plant-friendly" versions of cry6A by assembling the entire genes de novo from 70-90-mer oligonucleotides and high fidelity PCR. The synthesized cry6A gene driven by enhanced 35S promoter was introduced into tomato hairy root via Agrobacterium rhizogenes-mediated transformation. It took us four years to maximize expression level to 0.1% of total soluble protein with further extensive modification of the original synthesized cry6A gene. Although Northern blot and RT-PCR sequencing results showed the correct cry6A transcripts, several truncated protein bands are observed. We are currently testing the transgenic resistance of tomato hairy root lines to *Meloidogyne incognita*. Our progress in testing these lines for control of nematode infections will be reported.

REPORT OF *MELOIDOGYNE* SPP. IN PRESERVED AREAS OF ATLANTIC FOREST IN THE STATE OF RIO DE JANEIRO, BRAZIL. **Lima, Inorbert M., Ricardo M. Souza, Regina M. D. G. Carneiro, and Carina P. Silva.** Universidade Estadual do Norte Fluminense, Av. Alberto Lamego, 2000, Campos dos Goytacazes (RJ), Brazil.

The goal of this work was to identify *Meloidogyne* species in preserved areas of Atlantic Forest (A.F.) and "restingas" in the State of Rio de Janeiro, Brazil. A total of 350 samples, each composed of 2.5 liters of soil and roots, were taken from

the rizosphere of wood trees, palms, shrubs and herbaceous species in the localities of Terras Frias and Imbé (within the domains of the Desengano State Park), Serra da Sibéria (municipality of Nova Friburgo), Serra da Bicuda (municipality of Macaé), and in the Restingas de Jurubatiba National Park (municipalities of Quissamã and Carapebus). In the soil samples, tomato and okra were cultivated as bait-plants for 75 to 90 days, after which the root systems were checked for the presence of root galls and/or egg masses. Twenty-one *Meloidogyne* populations were recovered, being later purified through esterase isozyme electrophoresis of single nematode females and subsequent single egg mass inoculations. The taxonomic identification of the *Meloidogyne* populations was peformed by light microscopy examination of mature females, males and second-stage juveniles. In the A.F., 12 populations of *M. javanica* were recovered, as well as six of *M. exigua*, two of *M. incognita*, one of *M. arenaria*, one of *M. mayaguensis*, and one unindentified species. Most of the species were found isolated from others in the samples, with most of the populations being recovered from former A.F. in low and warmer areas. *M. javanica* was recovered in all areas, with the exception of Terras Frias, from which *M. exigua* only was recovered. For the first time in Brazil, *M. mayaguensis* was detected in a natural ecosystem, supporting the hypothesis that this highly damaging species is native, and not exotic, to Brazil. This work confirms the adaptation of *Meloidogyne* spp. to the A.F., and these species hold the potencial of adapting to crop plant species cultivated in former A.F. areas incoporated to Agriculture. *Meloidogyne* spp. was not recovered from the "restinga" ecosystem, suggesting that its environment is not suitable to this genus.

GENETIC OF ROOT-KNOT NEMATODE, *MELOIDOGYNE HAPLA*. **Liu, Qingli, and Valerie Williamson.** Department of Nematology, Plant Pathology Graduate Program, University of California, Davis, CA 95616.

Root-knot nematodes (*Meloidogyne spp.*) are obligate endoparasites whose genetics are poorly understood due to their complex reproductive modes. Although many agriculturally important species reproduce asexually, some strains of *M. hapla* undergo meiosis and sexual reproduction making them amenable to genetic crosses. We have five inbred strains of *M. hapla*. These strains have been inbred for 18 generations by single eggmass transfer on tomato. AFLP DNA fingerprinting revealed that DNA polymorphisms are very common among these strains. We have demonstrated that they represent the sexual form based on cytological studies. These strains also interact differently with the wild potato host *Solanum bulbocastanum*, in which VW9 and VW12 are virulent while VW8, VW10 and VW11 are avirulent. Attraction assays also showed VW9 is more attracted to the potato roots than VW8. In this study, we crossed female VW8 with male VW9. PCR-based markers in progeny revealed that the cross was successful. Segregation of the molecular markers in F2 populations suggests that *M. hapla* undergoes facultative meiotic parthenogenesis during its reproduction. We are currently producing additional molecular markers by AFLP DNA fingerprinting and we plan to generate a genetic linkage map for *M. hapla*.

EFFECT OF TILLAGE ON PARASITISM OF *HETERODERA GLYCINES* SECOND-STAGE JUVENILES BY *HIRSUTELLA* SPP. **Liu, S. F., and S. Chen.** Southern Research and Outreach Center, University of Minnesota, Waseca, MN 56093.

The effect of tillage on parasitism of *Heterodera glycines* second-stage juveniles (J2) by nematophagous fungi *Hirsutella minnesotensis* and /or *Hirsutella rhossiliensis* was investigated in four sites in soybean-corn rotation and one site in soybean monoculture in Minnesota. Soil samples were taken from two central rows each plot at planting, in midseason, and at harvest in 2002, and at planting, one and two months after planting, and at harvest in 2003. *Heterodera glycines* J2 were extracted, and percentage of J2 parasitized by *Hirsutella* were determined. No significant difference in percentage of J2 parasitized was observed between conventional tillage and no-till in all sampling occasions, sites, and both years except that the percentage of J2 parasitized in no-till was significantly higher than in conventional tillage at planting and at harvest in 2003 in one soybean field, which was in soybean-corn annual rotation. Nevertheless, percentage of J2 parasitized by *Hirsutella* was higher in soybean fields than in corn fields, and the percentage of J2 parasitized in midseason was higher than that at planting and harvest.

EFFECTS OF *BELONOLAIMUS LONGICAUDATUS* ON NITRATE LEACHING IN TURFGRASS LYSIMETERS **Luc, J. E., W. T. Crow, J. L. Stimac, J. B. Sartain, and R. M. Giblin-Davis.** University of Florida, Gainesville FL 32611.

Glasshouse experiments were conducted during 2002 and 2003 to quantify the relationship between root reductions of Tifdwarf bermudagrass caused by the sting nematode (*Belonolaimus longicaudatus*), and increases in the quantity of nitrate leached. Forty lysimeters in each of two trial were sprigged with Tifdwarf bermudagrass, of which 20 were inoculated with *B. longicaudatus* and 20 were uninoculated. The trials were initiated either 8 or 3 weeks after inoculation. Root length, surface area, and weight were compared between treatments at 6, 12, and 18 weeks after initiation of the trials. Turf was fertilized every 3 weeks and leaching events were simulated at 21 or 42-day intervals. The leachate was collected and the quantity of nitrate leached was compared between treatments. Root reductions were observed ($P < 0.05$) in lysimeters inoculated with *B. longicaudatus* at all evaluation dates and NO3- leached was greater ($P < 0.05$) in inoculated lysimeters at the 18 week evaluation during both trials. When amount of cumulative nitrate leached was compared between treatments,

lysimeters inoculated with *B. longicaudatus* leached more nitrate than did uninoculated lysimeters ($P = 0.05$) in trial 1 but only at ($P = 0.11$) in trial 2. Nematode feeding reduced root density by 30% to 94% and increased the amount of nitrate leaching as much as 429%. This study indicates that by damaging turf roots, *B. longicaudatus* may increase nitrate leaching, thereby adding to water quality concerns.

VARIABILITY AMONG GEOGRAPHIC ISOLATES OF *PRATYLENCHUS PENETRANS.* **MacGuidwin, A. E.,[1] R. C. Rowe,[2] and M. A. Omer.[2]** [1]Department Plant Pathology, University of Wisconsin, Madison, WI; [2]Department of Plant Pathology, Ohio State University OARDC, Wooster, OH.

Pratylenchus penetrans, in combination with the fungus *Verticillium dahliae*, causes the early dying disease of potato. Intraspecific variability in pathogenicity of *V. dahliae*, alone and in combination with *P. penetrans*, has been characterized. The purpose of this study was to determine if isolates of *P. penetrans*, could be distinguished by their ability to interact with *V. dahliae* or according to other biological criteria. Monoxenic cultures of *P. penetrans* on corn root explants were established from sixteen field populations of nematodes collected from Wisconsin, Ohio, Michigan, Minnesota, Washington, Oregon, and Canada. Potato plants grown in tissue culture were transplanted into pots filled with pasteurized Plainfield loamy sand soil and vermiculite (1:1 by volume) and infested with nematodes two weeks later. Verticillium was introduced into plants one week later by injecting conidia into one vascular bundle of potato stems using a hypodermic needle. The plants were grown in a growth chamber or greenhouse, scored by symptoms of potato early dying disease, and assayed to determine the extent of colonization by *V. dahliae* at the end of the study. All isolates of *P. penetrans* interacted with *V. dahliae*, but some accelerated the onset of symptoms or disease severity more than others. Several isolates were compared for their ability to induce glucanase in potato plants and differences were detected. Other attributes that varied among the isolates were sex ratio and reproduction on soybean.

INHIBITION AND RESTORATION OF EGG HATCH IN *HETERODERA GLYCINES.* **Masler, E. P,[1] P. A. Donald,[2] and S. Sardanelli.[3]** USDA ARS [1]Nematology Laboratory, Beltsville, MD 20705; [2]Crop Genetics and Production Research Unit, Jackson, TN 38301, and [3]Plant Nematology Laboratory, University of Maryland, College Park, MD 20742.

We are interested in the control of egg hatch by factors within cyst nematodes that either function autonomously or in response to external agents. Baseline *in vitro* hatching kinetics of *H. glycines* laboratory eggs were established using large (>10,000 eggs/assay) and small (500–1000 eggs/assay) scale systems. No differences in total percent hatch or in hatch rates were observed between the two systems, although there was some positive correlation between egg density and hatch rate. Hatching kinetics were then used to compare experimental treatments, and to compare hatch characteristics of laboratory eggs with those obtained from field samples. Hatching was retarded by mild rinsing of freshly collected eggs with tap water. This tap water rinse (TWR) was saved. Two days after rinsing, total percent hatch of the rinsed eggs was 90% lower than the total percent hatch of the non-rinsed control eggs. Seven days after rinsing, the eggs partially recovered, with hatch rates only 25% lower than the non-rinsed controls. Hatching could be completely restored in the rinse-retarded eggs by their exposure to the TWR used to rinse eggs. Eggs collected from field samples hatch at a very low rate under laboratory conditions (<0.1% per week). Following application of TWR to field eggs there was a distinct but small increase in hatch (0.5%–1.0% per day). The increase was not permanent and hatch rate fell to baseline level 2–3 days after TWR exposure. The factor(s) involved show some heat stability. These and other potential sources of hatch regulators within the nematode are discussed.

NOVEL NEMATICIDAL CHEMISTRIES IDENTIFIED THROUGH A COMPARATIVE AND FUNCTIONAL GENOMICS PLATFORM. **McCarter, James P., Deryck J. Williams, Andrew P. Kloek, Michelle C. Hresko, and Barry J. Shortt.** Divergence Inc., 893 North Warson Rd., St. Louis, MO 6314.

The goal of research at Divergence Inc. is the development of safe and effective nematode control technologies with novel modes of action. Based upon a comparative and functional genomics platform, Divergence has selected genes that are both specific and essential to nematodes. 1,200 genes were identified as conserved across the phylum yet divergent from the gene complement of mammals. C. elegans RNA interference demonstrated strong knock-out effects in 100 cases. The approach identified multiple essential biochemical pathways present in nematodes yet absent from vertebrates. Chemical DC7651 was selected based on similarity to the substrate of enzyme target DIV8338. DC7651 has demonstrated nematicidal potency against a wide array of nematodes including activity in greenhouse assays against the plant parasitic nematodes *Meloidogyne incognita, Heterodera glycines*, and *Belonolaimus longicaudatus* and in vitro against the mammalian parasites *Haemonchus contortus, Teladorsagia circumcincta, Trichostrongylus colubriformis, Brugia malayi*, and *Parastrongyloides trichosuri*. DC7651 is a synthetic derivative of a naturally occurring plant metabolite and lacks toxicity to rats, fish, earthworms, and insects at high doses suggesting that the compound specifically targets nematodes. Efforts are underway to optimize the potency and other properties of this lead chemistry. The identity of the DIV8338 target and DC7651 class of chemistry will be discussed.

CHEMICAL MANAGEMENT OF NEMATODES IN LOUISIANA: 2000–2003 FIELD AND MICROPLOT TRIALS WITH COTTON, SOYBEAN, SUGARCANE, RICE, AND ASSORTED VEGETABLES. **McGawley, Edward C.,[1] M. J. Pontif,[2] C. Overstreet,[3] Y. Li,[3] and J. B. Sumner.[4]** Department of Plant Pathology & Crop Physiology, Louisiana State University Ag Center, Baton Rouge, LA 70803.

Between 2000 and 2003, field trials with cotton, soybean and sugarcane were conducted to evaluate the efficacy of several labeled (Telone II [T—45.3 and 90.6 kg a.i./ha] and Methyl Bromide [MB—preplant—130.9 kg a.i./ha]) and one experimental (Agri-Terra, a colloidal suspension containing 1% monobasic sodium phosphate as the active ingredient [AgT—9.5 l/ha]) nematicides against nematode communities indigenous to Louisiana. Compared with controls at harvest across four years for cotton and soybean, labeled materials provided acceptable reniform and soybean cyst nematode control (population reductions of 28–55%) and yield response (increases of 8-30%). However, application of AgT produced reductions in nematode populations and increases in yield that were equal to and in most cases greater than these. A three-year-duration sugarcane trial, initiated in 2001 using the same treatments and an additional one (Agrizide [AgZ—AgT combined with 250mg/ml sodium azide—preplant—15, 30 or 45 mg/kg soil]) show similar trends of efficacy against nematodes and AgZ has produced encouraging results against weeds. In year one, there were no yield differences among treatments. Microplot trials were conducted using cotton, soybean, sugarcane, rice, tomatoes and pepper. Steam pasteurized soil infested with nematodes was used in 2000 and non-treated, naturally infested field soil was used in 2001 and 2002. Chemical treatments and controls were the same as those described for field trials with rates adjusted to 15 or 35 kg capacities of microplots. In all trials, AgT was equal to or better than labeled materials. Further application methodology/ crop tolerance work with AgZ is necessary.

THREE NEMATODE GENERA AND THE DAMAGE THEY CAUSE FOR PLUM PRODUCERS. **McKenry, Michael.** Kearney Horticultural Station, Parlier, CA 93648.

For fifty years Nemaguard (*Prunus persica* × *P. davidiana*) rootstock has provided relief from all *Meloidogyne* spp. in California. For 30 years two plum (*Prunus* spp.) rootstocks have also proven their resistance to *Meloidogyne* spp. Without this resistance California orchards could not be planted and replanted to coarse textured soils. Populations of *M. floridensis* reproduce on Nemaguard but their distribution is apparently limited to Florida. In California, Nemaguard rootstock can be severely damaged when planted into high population levels of *Meloidogyne* spp. Occasionally *Tylenchulus semipenetrans* can be found at low population levels on plum rootstocks but is not currently of concern there. *Pratylenchus vulnus* is present in 30% of California peach and plum acreage. *Mesocriconema xenoplax* occurs throughout California but damaging population levels occur only in highly porous soils. There is no commercial resistance to these latter two nematodes so prior to replanting, soils oftentimes require broadcast fumigation enabling one to six years of nematode relief. There is another factor common to old orchard soils that this author refers to as the rejection component of the replant problem. Solutions for the rejection component include strip fumigation or a fallow period that lasts four years. Along with factors such as soil texture, soil type, cropping history, rootstock selection, nematode species, irrigation method, and regional differences; initial tree establishment must be considered before estimates of crop damage by nematodes can reach perfection. Years of effort are needed to estimate nematode damage to plum, just one example of 200 "minor" crops produced in California. Long into the future crop loss estimates will continue to be just that, estimates.

REDUCING EXTRACTION BIAS IN NEMATODE COMMUNITY SAMPLES. **McSorley, R., J. J. Frederick, and K.-H. Wang.** Department of Entomology and Nematology, University of Florida, Gainesville, FL 32611-0620.

Methods for extraction of nematodes from soil samples vary in efficiency and may affect perception of nematode community structure. An active method (Baermann incubation) was compared with a passive method (centrifugation) for extraction of all taxa of the nematode communities in sandy soils in five experiments. Baermann incubation recovered only 30% of the herbivore numbers compared to centrifugation, but centrifugation recovered only 22% of the omnivores and 12% of the predators that were extracted by Baermann incubation. Centrifugation was consistently superior (higher recovery in at least 3 experiments) to Baermann incubation for recovery of *Acrobeles, Alaimus, Hemicriconemoides, Hoplolaimus, and Mesocriconema.* In contrast, Baermann incubation was consistently better than centrifugation for recovery of *Aphelenchoides, Eudorylaimus, Nygolaimus, Tylencholaimellus, Tylencholaimus*, and *Xiphinema*. Percentage composition of the nematode community was affected by extraction method, with a higher percentage of omnivores and predatores recovered by Baermann incubation and a higher percentage of herbivores recovered by centrifugation. A double extraction method (Baermann and centrifugation used independently) is proposed for each sample to optimize recovery of every taxon in the nematode community.

RESPONSE OF SELECTED *HETERODERA GLYCINES* POPULATIONS TO N-VIRO SOIL TREATMENT. **Melakeberhan, H.,[1] and G. R. Noel.[2]** [1]Department of Entomology, Michigan State University, East Lansing, MI 48824; [2]USDA/ARS, Department of Crop Sciences, University of Illinois, Urbana, IL 61801.

The worldwide geographic distribution of Heterodera glycines demonstrates broad adaptation to a range of environ-

mental conditions. In addition to environmental adaptation, H. glycines's high degree of parasitic variability presents multiple challenges for its management. However, it is unknown if H. glycines populations' parasitic variability is related to soil conditions. The purpose of this project was to test how H. glycines races 1, 2 and 3 respond to 0, 5 and 20 g of N-Viro Soil® (NVS) per 500 cm3 of sandy loam (87% sand, 8% silt and 5% clay, and pH 7.4) soil in two concurrently conducted greenhouse experiments (25 ± 2 oC) in 2002. NVS is a recycled municipal biosolid product used as a soil amendment in a range of conditions. Each pot, containing a 2-week-old H. glycines-susceptible Round-up Ready® soybean seedling (DSR-221, Dairy Land Seed Co.), was infested with either water (control) or 10,000 eggs consisting of 60 to 66% late pretzel (differentiated) stage of embryogenesis. Each experiment consisted of 48 units (4 nem × 3 NVS × 4 reps) and lasted 2 months. There was no clear effect on cyst development or eggs per cyst due to NVS treatment. However, the 20 g NVS/500 cm3 soil decreased the total numbers of H. glycines cysts and other life stages of all three populations in roots more than the other treatments in both experiments. Race 3 was least affected and race 1 the most affected by NVS treatment in both experiments. The results suggest that H. glycines populations differ in their adaptation to soil environments.

MORPHOLOGICAL AND MOLECULAR COMPARISONS BETWEEN *MONACROSPORIUM DRECHSLERI* AND RELATED NEMATODE-TRAPPING FUNGI. **Meyer, S. L. F.,[1] L. K. Carta,[1] and S. A. Rehner.[2]** [1]USDA ARS Nematology Laboratory, Beltsville, MD 20705; [2]USDA ARS Insect Biocontrol Laboratory, Beltsville, MD 20705.

Population densities of the nematode *Meloidogyne arenaria*, grown on tomato roots in the ARS Beltsville Nematology Laboratory greenhouse, declined after years of successful maintenance. Individual *M. arenaria* specimens from the pots were examined for fungal parasitism. The nematophagous fungus *Monacrosporium drechsleri* was subsequently isolated from second-stage juveniles of *M. arenaria*. Although no individuals of the free-living *Diploscapter* spp. observed from the same pots were visibly parasitized, adhesive knobs formed by cultures of the *M. drechsleri* isolate trapped all genera of nematodes tested in Petri dish cultures, including the plant parasites *Heterodera glycines*, *Meloidogyne incognita*, *Pratylenchus zeae*, and the free-living nematodes *Caenorhabditis elegans* and *Panagrellus redivivus*. To examine relationships of *M. drechsleri* with other *Monacrosporium* species, the Beltsville isolate was compared with the morphologically similar species *M. ellipsosporum* and *M. lysipagum*, and with the less similar species *M. parvicolle*. The ITS-1 and -2 regions of rDNA and the nuclear gene *EF1-apha* were sequenced for all four species. Parsimony trees indicated that the closest molecular relative of *M. drechsleri* was *M. ellipsosporum;* however, the tested *M. ellipsosporum* isolate (ATCC 204100) had a highly divergent sequence from that previously recorded in GenBank for a different isolate morphologically identified as *M. ellipsosporum* (CBS 224.54, GenBank accession U51971). The morphological characters can be difficult to use in separation of some of these taxa, demonstrating the usefulness of molecular characters for identification.

A LIST OF EXOTIC NEMATODE PLANT PESTS OF AGRICULTURAL AND ENVIRONMENTAL SIGNIFICANCE TO THE UNITED STATES. **Millar, L.,[1] P. Lehman,[2] R. Inserra,[2] T. Powers,[3] J. Brito,[2] K. Dong,[4] and Z. Handoo.[5]** [1]USDA-APHIS, Raleigh, NC 27606; [2]Florida Department of Agriculture and Consumer Services, Gainesville, FL 32614-7100; [3]Plant Science Department of Plant Pathology, University of Nebraska-Lincoln , Lincoln, NE 68583; [4]California Department of Food and Agriculture, Sacramento, CA 95832-1448, 5USDA, ARS, Beltsville, MD 20705.

Prioritization of exotic plant pest threats is an important step in mitigating the adverse effects of invasive plant pests to US agricultural and natural ecosystems. In particular, this prioritization improves the ability to identify emerging pest threats and to more quickly respond to new introductions. In order to assist the national initiative in regard to invasive nematode plant pests, in 2001, the Society of Nematologists (SON) signed and initiated a cooperative agreement with the US Department of Agriculture's Animal and Plant Health Inspection Service (APHIS) to develop a list of the most significant exotic nematode pests not present or of limited distribution in the US. The SON set up a Regulatory Committee working group of six member nematologists with expertise in the relevant areas of risk assessment, nematode detection and identification, and regulatory nematology. This working group developed a preliminary approach to identifying and ranking exotic nematode plant pests. Three prioritized lists were prepared. Fact sheets were included for each nematode on the lists. These project results were published on the internet (http://nematode.unl.edu/projectpest.htm). Only plant nematode pests which currently are or could potentially be APHIS quarantine pests were considered for the list. There are currently 49 nematode species listed. An overview of the process used to develop the nematode pest lists, the updated lists, and a discussion of the current and future applications of this initiative are presented.

THE EYE AND PHOTORECEPTORS OF *MERMIS NIGRESCENS*. **Mohamed, Abir, and A. H. Jay Burr.** Department of Biological Sciences, Simon Fraser University, Burnaby, BC V5A 1S6 Canada.

The eye used by the female *Mermis* as she climbs through vegetation toward egg-laying sites in grass is unique. Rather than the pair of melanin-like pigment spots or cups of other nematode eyes, the shadowing structure consists of crystalline hemoglobin in a large hollow cylindrical structure formed by projections of hypodermal cords. Previous experiments on the phototaxis of *Mermis* have strongly indicated that a photosensory cell must be located within the hollow cylinder of

pigment. In the several other types of nematode eye, a multilamellar or multiciliary dendritic process can be easily identified as a photoreceptor because of its appropriate close proximity to the shadowing pigment. However, in the *Mermis* eye a photoreceptor has been difficult to find. We here provide a reconstruction of the eye based on serial sections and TEM. The pigmented structure begins approximately 80 μm posterior to the tip and is approximately 106 μm in diameter and 250-300 μm long. The two amphidial and four cephalic nerve tracks pass through the cylinder. Two multilamellar sensory processes are located near the anterior opening of the pigment cylinder where they would be exposed to light from the anterior direction. Each lies within one of the amphidial tracks where they are unlikely to have another sensory function.

Because of their typical nematode sensory structure, the extended membrane surface area expected of photoreceptors, and their location where predicted by our behavioral experiments, we conclude these are likely the photoreceptors involved in the phototaxis.

UTILIZING SPATIALLY DERIVED DATA FOR MODELING *MELOIDOGYNE INCOGNITA, THIELAVIOPSIS BASICOLA,* AND INFLUENTIAL SOIL FACTORS ON YIELD. **Monfort, W. S.,[1] T. L. Kirkpatrick,[1] C. Rothrock,[2] and A. Mauromoustakos.[1]** Dept. of Plant Pathology, University of Arkansas, Fayetteville, AR 72701; and SWREC, Hope, AR 71801.

Meloidogyne incognita and *Thielaviopsis basicola* population densities and soil factors that influence their densities and distribution were evaluated in 2001 and 2002 in a commercial cotton field in southeastern Arkansas to evaluate their effects on yield. The 6-hectare field was subdivided into 512 grid plots (30.5 meters × 3.9 meters) and sampled for *M. incognita* in April, May, July, and October. April and October samples were also assayed for *T. basicola*. Soil fertility at planting and soil texture was also determined from each grid plot. Fumigant nematicide, 1,3-dichloropropene (Telone II) was applied in strips through the field at 14.2, 28.4, and 42.6 liters/ha. to create zones within the field with differential population densities of the nematode. Statistical analyses were conducted in JMP SAS software utilizing stepwise and multiple regression. Variables that were important in explaining cotton yield differences were percent sand, nematicide application, *M. incognita* (April 2002 density), and *T. basicola* (October 2001 density), although only <30% of the variability was accounted for. However, when the individual grid plots were aggregated based on four arbitrarily selected soil textural ranges (0–30% sand, 31–45% sand, 46–60% sand, and >60% sand), and analyzed based on the nematicide treated zones within each textural class, 89% of the yield variability was accounted for in 2002. These findings indicated that soil texture might have a significant impact on the damage potential of *M. incognita* and *T. basicola* on cotton and may need to be accounted for when evaluating either of these pathogens in the field.

RESISTANCE TO *ROTYLENCHULUS RENIFORMIS* IN INTERSPECIFIC *GOSSYPIUM* HYBRIDS. **Moresco, E.,[1] E. Morgan,[2] K. W. Ripple,[1] C. W. Smith,[1] and J. L. Starr.[2]** [1] Department of Soil and Crop Sciences, Texas A&M University, College Station, TX 77843; [2] Dept Plant Pathology and Microbiology, Texas A&M University, College Station, TX 77843.

Rotylenchulus reniformis is increasing in importance as a yield-limiting pathogen of cotton in several cotton production areas in the United States. Currently, no genotype of upland cotton that has a high level of resistance to this nematode is known. *Gossypium barbadense* 'Tx110', which has moderate resistance to *R. reniformis*, was crossed with *G. hirsutum* 'M315'. Progeny of the cross were selected for flowering below node 9 in the F2 generation, and for resistance to *R. reniformis* based on inhibition of nematode reproduction in the F3 and F4 generations. Evaluation of several F5 lines indicates that resistance is a fixed trait, with these lines supporting nematode population densities that ranged from 4% to 13% of that on the susceptible M315. Resistance in Tx110 is expressed as fewer nematodes invading the roots and taking greater time to develop to maturity, fewer eggs per g roots, and fewer eggs per female.

IMPROVING ESTIMATES OF NEMATODE-INDUCED DAMAGE IN COTTON AND SOYBEAN. **Mueller, J. D.,[1] and T. L. Kirkpatrick.[2]** [1]Clemson University, Edisto Research and Education Center, Blackville, SC 29817; [2]University of Arkansas, Southwest Research and Education Center, Hope, AR 71801.

Yield loss estimates in cotton and soybean have traditionally been generated by visual "surveys" of the losses incurred each year. These have been maintained for many years by groups such as the Cotton Disease Council and the Southern Soybean Disease Workers. These losses are often reported as dollars lost and even with a small percentage loss the overall dollar figures are quite high. Today there is a great need for more accurate estimates of yield losses not only across large acreages but also to predict yield loss potentials of specific fields. Estimates across large acreages are needed to enable private industries to do cost-benefit analyses prior to beginning the process of bringing a product to market. New developments in precision agriculture and nematicide delivery are changing application methods; subsequently, the cost for applications on a given acreage have changed. Growers need to be able to accurately estimate the yield loss potential for individual fields or subunits to evaluate the potential benefits of treatment options. Another developing change in nematode management in cotton and soybean is the development of cultivars with high levels of resistance to Meloidogyne incognita

and other nematode species. Using a combination of resistant cultivars and improved nematode distribution maps will give us our best estimates yet of the real levels of yield losses occurring in fields.

AN 18S DNA BARCODE APPLIED TO NEMATODES FROM THE KONZA TALLGRASS PRAIRIE. **Mullin, P. G., T. S. Harris, R. S. Higgins, and T. O. Powers.** Department of Plant Pathology, University of Nebraska, Lincoln, NE 68583.

The Konza Tallgrass Prairie, located in the Flint Hills of eastern Kansas, is exceptionally rich in nematode species diversity. An estimate of taxonomic diversity based on morphology inventoried 356 species along 100 m of an upland ridge. Representative morpho-species found at a frequency of 0.2% or greater in the survey were individually selected for PCR amplification of an approximately 650-bp 3′ fragment of the 18S ribosomal DNA. Sequences have been compiled for 243 specimens representing 145 nominal species based on morphological identification. Overall there was good agreement between estimates of species diversity predicted from morphology versus DNA sequence. Sequences were obtained from multiple specimens of fifty-four of the identified species. In some genera, *Aphelenchus, Ditylenchus, Eucephalobus* and *Eudorylaimus*, for example, the DNA barcode revealed additional unrecognized diversity within species. In other genera, such as *Aporcelaimellus*, morphologically distinct nominal species had identical barcode sequences. The barcode was particularly useful for identification of juveniles, dauer stages, and other specimens for which standard keys are inadequate.

PHYLOGENETIC RELATIONSHIPS OF NYGOLAIMINA AND DORYLAIMINA (NEMATODA: DORYLAIMIDA) INFERRED USING SMALL SUBUNIT RIBOSOMAL DNA. **Mullin, P. G., T. S. Harris, and T. O. Powers.** Department of Plant Pathology, University of Nebraska, Lincoln, NE 68583.

Phylogenetic reconstructions based on 18S rDNA sequence data indicate that Dorylaimida, comprising the suborders Nygolaimina and Dorylaimina, is a monophyletic lineage, but that there is a deep division within Nygolaimina, giving rise to the possibility that Nygolaimina is paraphyletic. A well-supported clade comprising members of the traditional orders Mermithida and Mononchida (including Bathyodontina) forms the sister taxon to the Dorylaimida. Inferred relationships within this clade suggest that either Bathyodontina should be raised to ordinal status or that Mermithida should be demoted to a suborder of Mononchida. Vertebrate parasites within Dorylaimia (Dioctophymida and Trichinellida) are reconstructed in a sister-taxon relationship with the Mononchida/Dorylaimida lineage. The enigmatic order Isolaimida (represented by *Isolaimium*) appears to be ancestral to all other Dorylaimia sampled. This indicates that, in contrast to recent phylogenetic hypotheses for this group, the common ancestor of Dorylaimia may have lacked a spear and possessed pharyngeal gland outlets posterior to the nerve ring, rather than in the stomatal region.

IMPACT OF COLEOPTERAN-ACTIVE BT CORN ON NON-TARGET SOIL NEMATODE COMMUNITIES. **Muthumbi, Agnes,[1,2] and D. A. Neher.[1]** [1]Department of Earth, Ecological and Environmental Sciences, University of Toledo, Toledo, OH 43606; [2]Department of Zoology, University of Nairobi, P.O. Box 30197, Nairobi, Kenya.

Nematodes reside in the rhizosphere and are potentially exposed to Cry3Bb1 toxins exudated from roots of Bt corn (event MON863) targeted for corn rootworms. A Bt hybrid was compared to 1) a non-Bt, isogenic hybrid treated with a soil insecticide, and 2) a non-Bt, isogenic hybrid without insecticide. Treatment plots (0.2 ha) were established in a non-till field and replicated three times in a Latin square design. Nematodes were extracted, from soil samples collected prior to planting (May), at peak anthesis (August), and after harvest (October) in 2003, enumerated and identified to genus. Overall numbers of nematodes were greater in May and October than August. Sixty-eight genera from 34 families were identified. The most abundant genus was Tylenchorynchus, which accounted for 25% of the total abundance. Maturity, channel, enrichment and structure indices indicated no differences between Bt and non-Bt treatments. Trophic composition of communities was similar except for the proportion of omnivores, which was different significantly among treatments. Trophic and generic diversities were greater in Bt than non-Bt communities. In contrast, abundance of nematode genera was distinctly different among corn hybrids, quantified by multivariate canonical correspondence analysis and principal response curves. Non-target impacts of Bt corn on nematode communities were apparent at the genus level but community structure or successional stage were unaffected.

MOLECULAR PHYLOGENETIC EVIDENCE SUPPORTS MONOPHYLY OF THE CEPHALOBOIDEA BUT NOT THE PANAGROLAIMOIDEA.

Nadler, Steve,[1] P. De Ley,[2] I. T. De Ley,[2] M. Mundo-Ocampo,[2] A. B. Smythe,[1] D. J. Bumbarger,[2] and J. G. Baldwin.[2] Departments of Nematology, [1]University of California, Davis, CA 95616; [2]University of California, Riverside, CA 92521.

Nematodes of the suborder Cephalobina represent a morphologically and ecologically diverse set of taxa that includes free-living microbivores, insect associates, entomopathogens, and opportunistic parasites of vertebrates. A molecular phylogenetic hypothesis was developed for Cephalobina using nuclear-encoded large subunit (LSU) ribosomal DNA sequences (2,707 characters). These sequence data were obtained for 47 Cephalobina representing 31 genera, including 29

Cephaloboidea and 18 Panagrolaimoidea. Trees were inferred by maximum parsimony and likelihood methods, and robustness assessed by bootstrap resampling. These analyses yielded strong support for monophyly of the Cephaloboidea, but indicate that certain genera of Cephalobidae (e.g., *Acrobeles, Cervidellus*) are not monophyletic. Panagrolaimoidea was not monophyletic, although a clade consisting of seven panagrolaimid genera was strongly supported. Strongyloidoidea, represented by *Rhabditophanes, Strongyloides,* and *Steinernema* was not monophyletic, and the steinernematids unexpectedly grouped with the rhabditoid outgroups. Monophyly of representative Chambersiellidae was strongly supported, but the position of chambersiellids, *Myolaimus,* and representative Tylenchida were not well resolved in the LSU sequence tree.

QUANTITATIVE TOOLS FOR DETECTION OF SENTINEL TAXA AMONG COMPLEX DATA. **Neher, Deborah A.** Department of Earth, Ecological and Environmental Sciences, University of Toledo, Toledo, OH 43606.

Nematode community indices would be made more feasible for use in national or regional environmental monitoring programs by reducing the number of genera that need to be enumerated and identified. This could be achieved by narrowing indices to include only sensitive or tolerant taxa that have distinctive and predictable responses to a specific type of disturbance while eliminating ambiguous ones. Disturbances can be classified as biological, physical or chemical. Multivariate statistics provides a cost-effective tool to identify sentinel taxa from existing and complex data that include numbers of nematode genera or species, land management practices, land use history, and soil properties. Canonical correspondence analysis (CCA) and partial CCA can be used to segregate direct and indirect effects of a disturbance type. Principle response curves can help identify temporal relations. Soil chemical and physical properties directly affect habitat and, thus indirectly influence nematode community composition. These factors can be treated as covariables to quantify their contribution to community variation. Once taxa are chosen, their sentinel status can be verified and validated by subsequent experiments designed to control type and magnitude of disturbance. Ideally, sentinel taxa response will withstand local variability in vegetation, soil type and microclimate. Narrowing the list of taxa for inclusion in nematode community indices will improve their calibration and interpretation, and make possible the establishment of a molecular-based toolkit available to nonspecialists.

THE IMPORTANCE OF TROPICAL ROOT-KNOT NEMATODES (*MELOIDOGYNE INCOGNITA*) AND STEM-ROT DISEASE (*SCLEROTIUM ROLFSII*) ON TOMATO CULTIVARS. **Ngo, Xuyen Thi.** Department Plant Pathology, Hanoi Agricultural University, Gialam, Hanoi, Vietnam.

Most tomato cultivars planted in North Vietnam represent all the possible combinations of susceptibility to root-knot nematodes *(Meloidogyne incognita)* and stem-rot disease *(Sclerotium rolfsii)*. This disease combination was the most widely distributed field disease of tomato plants. The presence of *M. incognita* contributed to an early onset and increased severity of *Sclerotium rolfsii* symptoms and plant stunting. Root galling, egg masses and density of second-stage larvae of *M. incognita* were generally more increased by 20%, 8–22%, and 7% on a combination treatment *M. incognita* and *Sclerotium rolfsii* than when *M. incognita* was used alone. The tomato cultivars of VFN-Roma, Motell, HT-7, VL-2000 were resistant to the tested *M. incognita* and *Sclerotium rolfsii* isolates.

RESPONSE OF SOIL NEMATODES TO MOISTURE ADDITIONS FROM SNOW FENCES IN TAYLOR VALLEY, ANTARCTICA. **Nkem, J. N,[1] D. H. Wall,[1] R. A. Virginia,[2] E. J. Broos,[1] J. E. Barett,[2] B. J. Adams,[3] and A. N. Parsons.[1]** [1]Natural Resource Ecology Laboratory, Colorado State University, Fort Collins, CO 80523; [2]Environmental Studies Program, Dartmouth College, Hanover, NH 03755; [3]Microbiology and Molecular Biology, Brigham Young University, Provo, UT 84602.

Taylor Valley is a polar desert with very low soil moisture content (~1%) and nematode diversity (3 species). Changes in soil moisture associated with climate variation may influence nematode populations over time. We erected snow fences 1 m high and 6 m long in two lake basins; Fryxell and Bonney, to evaluate soil nematode response to moisture addition from melt of drifted snow accumulated on the leeside of the fences. Soil samples were taken after snow melt at 0.91 m intervals to a distance 3.65 m from the snow fence in the austral summer of 2001, 2003, and 2004, and analyzed for nematode response and moisture content. Soil moisture differed over time and between the two basins ($P < 0.0001$) but was not different between the sampling positions. Fryxell, located closest to McMurdo Sound, had higher moisture than Bonney and there was a significant increase in soil moisture content in 2004. Soil nematode abundance declined over time ($P < 0.0001$) especially with increasing soil moisture (>2%) but species composition did not change. Species response to increasing soil moisture differed. Abundance of the dominant species, Scottnema lindsayae declined ($P < 0.0001$) while the abundance of Plectus antarcticus increased ($P < 0.0155$) and Eudorylaimus antarcticus showed an increasing trend. Total nematode mortality increased over time mostly due to Scottnema lindsayae ($P < 0.0001$), which responded negatively to soil moisture increases. Long-term supplements of moisture to soils could potentially change the community structure and population dynamics of soil nematodes in the dry valleys.

HETERODERA GLYCINES POPULATION DEVELOPMENT ON SOYBEAN TREATED WITH GLYPHOSATE. **Noel, G. R., and L. M. Wax.** USDA- ARS, and Department of Crop Sciences, University of Illinois, Urbana, IL 61801.

A concern when planting genetically modified crops is the potential effect of the technology on nontarget organisms. Glyphosate translocates to the soybean root where the herbicide might affect the biology of *Heterodera glycines*. In 2002 and 2003 Roundup Ready® soybean DSR 320 susceptible to *H. glycines* and DSR 327 resistant to *H. glycines* were grown in field plots in two different fields and either sprayed with glyphosate at the recommended rate and time of application or not sprayed. The experiment was arranged in a randomized complete block design with 12 replications. Nematode reproduction (Pf/Pi) and soybean yield were determined. In both years Pi was below the damage threshold, ranging from 72 to 205 eggs/100 cm^3 soil in 2002 and from 16 to 133 eggs/100 cm^3 soil in 2003. In both years Pf/Pi was greater $P <$ .0001) on DSR 320 when compared with DSR 327. Differences in Pf/Pi between DSR 320 treated or not treated with glyphosate were significant at $P = 0.08$ in both 2002 and 2003. There were no significant differences in Pf/Pi between DSR 327 treated with glyphosate or not treated. Soybean yield was not affected either by *H. glycines* or treatment with glyphosate. Whether or not glyphosate application increases populations of *H. glycines* is not clear. Additional studies in fields with larger numbers of *H. glycines* at planting are needed.

INCIDENCE AND ASSOCIATION OF *MELOIDOGYNE PARTITYLA* WITH MOUSE-EAR DISORDERS OF PECAN IN GEORGIA. **Nyczepir, A. P., C. C. Reilly, and B. W. Wood.** USDA-ARS, SE Fruit & Tree Nut Research Laboratory, 21 Dunbar Rd., Byron, GA 31008.

The pecan root-knot nematode, *Meloidogyne partityla* (Mp), has been associated with pecan trees exhibiting aboveground symptoms that included dead branches in the upper canopy, severely stunted growth, and (or) mouse-ear leaf symptoms. In 2003-04, a survey was conducted in the major pecan growing regions of Georgia to determine distribution of Mp and other *Meloidogyne* spp. Root samples infested with *Meloidogyne* spp. were obtained from 13 different pecan production sites. Female nematodes were teased from fresh root galls of declining trees and identified by determining the esterase phenotype from replicate samples of single females as compared to standard root-knot nematode species. *Meloidogyne partityla* and two unknown *Meloidogyne* spp. were the only root-knot nematode species found parasitizing pecan. *Meloidogyne partityla* was found in a greater number of samples and appears to be the dominant root-knot nematode species in pecan. In July 2002, the relationship between Mp alone and in combination with *Mesocriconema xenoplax* (Mx) on incidence of mouse-ear (ME) was initiated in field microplots. The occurrence of ME symptoms was detected at bud break on foliage in April 2003, nine months after inoculation. Trees were rated for ME leaflet deformity and severity. The ME severity index consisted of a 1-to-10 scale, with 1 = no ME leaflet symptoms and 10 = >50% leaf distortion and multiple stunted shoots. Trees growing in Mp-infested soil alone (8.3) and Mp + Mx infested soil (7.6) exhibited greater ($P < 0.05$) ME symptoms than in the uninoculated control (4.6) plots. No differences in degree of ME severity occurred between Mx-infested soil alone (5.9) and the uninoculated control plots.

APPLICATION OF DIVERSITY AND ECOLOGY INDICES TO EVALUATE NEMATODE COMMUNITY CHANGES AFTER FUMIGATION. **Okada, H.,[1] H. Harada,[2] and I. Kadota.[3]** [1]Nematology and Soil Zoology Unit, National Institute for Agro-Environmental Sciences, Kan'nondai 3-1-3, Tsukuba, Ibaraki, 305-8604; [2]Nagareyama, Chiba, 270; [3]Laboratory of Crop Protection, National Agricultural Research Center for Tohoku region, Arai, Fukushima, Fukushima, 960-2156, Japan.

Soil nematode density and family-level composition were investigated for five months after chloropicrin fumigation in soybean fields in Fukushima, northern Japan, to examine whether diversity and ecology indices can describe appropriately structural changes in nematode communities after chemical disturbance. Total nematode density in fumigated plots drastically decreased just after chloropicrin treatment, but gradually recovered. In fumigated plots after the treatment, the dominant nematode taxon changed from Rhabditidae to Cephalobidae; fungivorous guild with cp value 2 (Fu2) and guilds with cp 3-5 (CP35) were hardly detected. Whereas in untreated plots, Rhabditidae still predominated; Fu2 and CP35 increased. Among the diversity and ecology indices examined, Shannon-Weaver index of diversity, Maturity Index (MI), Maturity Index without opportunistic bacteriovorous guilds (MINO), Enrichment (EI) and Structure Index (SI) succeeded to describe these structural changes in nematode communities. Channel Index failed to detect significant effect of fumigation, because both of Fu2 and Rhabditidae reduced in fumigated plots. Replacement of Rhabditidae with Cephalobidae in the plots required careful interpretation of MI and EI behaviors. Even under such a replacement, MINO and SI were considered to be able to evaluate appropriately ecological status of nematode communities after chemical disturbances.

IDENTIFICATION OF MICROORGANISMS INVOLVED IN SOIL SUPPRESSIVENESS AGAINST THE PLANT-PARASITIC NEMATODE, *HETERODERA SCHACHTII*. **Olatinwo, R. O., B. Yin, J. O. Becker, and J. Borneman.** University of California, Riverside, CA 92521.

An approach was developed for identifying microorganisms involved in soil suppressiveness. Phase one of the approach employed an rRNA gene (rDNA) method to identify fungi and bacteria that positively correlated with soil suppressiveness against the plant-parasitic nematode, *Heterodera schachtii*. Five soil treatments with various levels of suppressiveness, generated by mixing different amounts of suppressive and fumigation-induced non-suppressive soil, were infested with *H.*

schachtii juveniles and cropped with Swiss chard. Since *H. schachtii* cysts isolated from this suppressive soil can transfer the suppressiveness to non-suppressive soil, these studies examined the cyst-associated microflora instead of the more complex soil communities. After two nematode generations, the most abundant fungal and bacteria rDNA sequences found in *H. schachtii* cysts isolated from the highly suppressive soils had high identity to *Dactylella oviparasitica* and *Zoogloea* sp. rDNA, respectively. The most abundant fungal rDNA sequences found in *H. schachtii* cysts isolated from the minimally to moderately suppressive soils had high identity to *Fusarium oxysporum* rDNA. Phase two confirmed these population trends using sequence-selective quantitative PCR. In phase three, strains of *D. oviparasitica*, *F. oxysporum* and the *Zoogloea* sp. were isolated from *H. schachtii* cysts and reintroduced into fumigation-induced non-suppressive soil. Individual introduction of *F. oxysporum* and the *Zoogloea* sp. did not significantly decrease *H. schachtii* populations, while *D. oviparasitica* decreased *H. schachtii* populations to levels equivalent to that produced by the suppressive soil after two nematode generations. The combined introduction of all three microorganisms reduced the level of *H. schachtii* juveniles below that observed in the suppressive soil. This general experimental approach should be useful for investigations of other suppressive soils and for identifying microorganisms involved in other functions.

DEVELOPMENT OF SPECIES-SPECIFIC PRIMERS FOR THE ECTOPARASITIC NEMATODE SPECIES *XIPHINEMA BREVICOLLE*, *X. DIFFUSUM*, *X. ELONGATUM*, *X. IFACOLUM*, AND *X. LONGICAUDATUM* (NEMATODA: LONGIDORIDAE). **Oliveira, C. M. G.,[1,2] B. Fenton,[1] G. Malloch,[1] D. J. F. Brown,[3] and R. Neilson.[1]** [1] Scottish Crop Research Institute, Dundee, DD2 5DA, Scotland; _Instituto Biológico, P.O. Box 70, 13001-970 Campinas, SP, Brazil; [3]Central Laboratory of General Ecology, 1113 Sofia, Bulgaria.

Single-step PCR species-specific diagnostic primers were developed that reliably discriminates four economically important *Xiphinema* species (*X. brevicolle*, *X. elongatum*, *X. ifacolum* and *X. longicaudatum*) and *X. diffusum* that is taxonomically very similar to *X. brevicolle*. Each species-specific reverse primer was located in the ITS-1 rDNA region and used in combination with an universal forward primer located in the 18S rDNA gene. Primer reliability was confirmed by screening different populations of the target species. Specificity was demonstrated by the absence of cross-reactions with 14 non-target *Xiphinema* species. Multiplex PCR was effective and reproducible for either two (*X. ifacolum* and *X. longicaudatum*) or three (*X. brevicolle*, *X. diffusum* and *X. elongatum*) target nematode species, thus improving the applicability of the diagnostic primers.

MOLECULAR AND MORPHOMETRIC ANALYSES OF *XIPHIDORUS* SPECIES (NEMATODA: LONGIDORIDAE). **Oliveira, C. M. G.[1,2] L. C. C. B. Ferraz,[3] A. R. Monteiro,[3] B. Fenton,[1] G. Malloch,[1] and R. Neilson.[1]** [1] Scottish Crop Research Institute, Dundee, DD2 5DA, Scotland; [2] Instituto Biológico, P.O. Box 70, 13001-970 Campinas, SP, Brazil; [3] ESALQ/USP, P.O. Box 09, 13418-900, Piracicaba, SP, Brazil.

Xiphidorus nematodes are indigenous to Latin America and have a restricted geographical distribution as compared with *Xiphinema*. A principal component analysis (PCA) based on 11 morphometric characters from 39 South America populations of *Xiphidorus* clearly separated populations previously identified as *X. achalae*, *X. amazonensis*, *X. minor*, *X. saladillensis*, *X. uruguayensis* and three undescribed *Xiphidorus* species. However, populations identified as *X. balcarceanus*, *X. parthenus* and *X. yepesara* did not form similar discrete groupings and exhibited either considerable morphological variability or have been incorrectly identified. Although not congruent, maximum likelihood phylogenetic trees derived from both 18S rDNA gene and ITS-1 sequences and digestion of PCR products derived from the ITS-1 region using three restriction enzymes (Taq I, Rsa I and Hinf I) discriminated six *Xiphidorus* species (*X. balcarceanus*, *X. minor*, *X. parthenus*, *X. yepesara*, and two undescribed *Xiphidorus* species) from Brazil. Sequence divergence was noted between populations of *X. parthenus* and *X. yepesara*. Our morphometric and molecular data strongly suggests that *X. parthenus* and *X. yepesara* are distinct taxonomic species contrary to their previous subspecies status and synonymization.

DEVELOPING MANAGEMENT ZONES FOR ROOT-KNOT NEMATODE IN COTTON IN MISSISSIPPI ALLUVIAL SOILS.

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A study is being evaluated to define nematode management zones using bulk soil electrical conductivity (SEC) as a surrogate for soil texture. Many fields along the Mississippi River have wide ranges of soil texture (sandy loams to clays) and have root-knot nematode as a primary pest along sandy ridges or portions of these fields. SEC was found to be highly correlated ($r = 0.94$) with soil clay content when mapped with a Veris 3100 Soil EC Mapping System. Two fields located in the Mississippi River alluvial soil area of Louisiana were selected that had a wide range of soil texture and known infestation of the Southern root-knot nematode (*Meloidogyne incognita*). Each field was mapped with the Veris system, which acquires SEC data at 1-second intervals and geo-references the data using a DGPS receiver. The Ken's Corner field (located in Tensas Parish) was 33.2 ha in size and was classified into 10 zones based on SEC reading (mS/m). The Roger

Carter field (located in Concordia Parish) was 24.4 ha in size and was classified into 8 zones. Nematode samples were collected from 0.4 ha grids and representative of the dominant SEC reading in that grid. Root-knot nematode was found at potentially damaging levels in the zones with the lowest SEC reading (zones 1–3 for the Carter field and zones 1–4 for the Corner field). Management zones could be developed to treat only areas where the root-knot nematode was a potential problem. Nematicide application could be reduced by 59% at the Tensas Parish site and by 62% at the Concordia Parish site.

INFLUENCE OF *ROTYLENCHULUS RENIFORMIS* ON SEEDLING DISEASE PATHOGENS OF COTTON. **Palmateer, A. J.,[1] and K. S. Lawrence.[2]** [1]Tropical Research & Education Center 18905 S.W. 280 St. Homestead, FL 33031; [2]Department of Entomology and Plant Pathology, Auburn University, Auburn, AL 36849.

Influence of *Rotylenchulus reniformis* in association with ten *Fusarium* species on cotton seedling disease was examined. Fungal treatments were *Fusarium chlamydosporum, F. equiseti, F. lateritium, F. moniliforme, F. oxysporum, F. oxysporum* f.sp. *vasinfectum, F. proliferatum, F. semitectum, F. solani,* and *F. sporotrichioides, Rhizoctonia solani,* and *Thielaviopsis basicola.* The experimental design was a 2 × 14 factorial in the presence or absence of *R. reniformis* and the 12 fungi plus two controls in autoclaved soil. In experiment 2, the design was a 2 × 2 × 14 factorial with the same treatments in autoclaved and non-autoclaved soil. *Fusarium oxysporum* f. sp. *vasinfectum, F. solani, R. solani*, and *T. basicola* displayed extensive root and hypocotyl necrosis that was more severe in the presence of R. reniformis. Soil treatment influenced the impact of *Fusarium* species on disease which was more severe in autoclaved soil. *Rotylenchulus reniformis* reproduction was greater in non-autoclaved soil compared to autoclaved soil ($P < 0.05$).

INFECTION DYNAMICS OF THREE ENTOMOPATHOGENIC NEMATODE SPECIES. **Perez, E. E., and E. E. Lewis.** Entomology Department, Virginia Polytechnic Institute & State University, Blacksburg, VA 24061.

We measured daily fluctuations in infection rates of *Steinernema feltiae*, *S. carpocapsae*, and *S. glaseri* infective juveniles (IJs) into *Galleria mellonella* larvae as hosts in two ways. In the first experiment, hosts were exposed to either IJs of *S. glaseri* or *S. feltiae* in sand. After 24 h of exposure, hosts were exposed to 100 naïve IJs of the same species. This process was repeated daily until IJs began to emerge from the host. Infective juveniles remaining in the sand were counted and the number of IJs entering the host was calculated. Infective juveniles penetrated hosts during the entire course of the experiment. The total number of infecting IJs was lowest for hosts infected with *S. glaseri*. For *S. glaseri* and *S. feltiae*, at both initial infection rates, the highest infection levels occurred during the first day of exposure and the number of IJs joining the infection declined linearly for the remainder of the experiment. In a second experiment, we exposed hosts to 300 IJs of *S. feltiae*, *S. carpocapsae* or *S. glaseri* for different durations, the shortest being 2 hrs and the longest 12 days. Using the same methods as described above, we found that approximately 90% of the maximum infection rates for each species were reached within 24 hours of initial exposure. Taken together, the results of these experiments suggest that the age of an infection affects the attractiveness of that resource for IJ entomopathogenic nematodes.

SUPPRESSION OF PLANT-PARASITIC NEMATODES IN TURFGRASS AFTER APPLICATION OF BIOLOGICALLY-BASED TREATMENTS**.** **Perez, E. E., and E. E. Lewis.** Entomology Department, VPI & SU, Blacksburg, VA 24061.

A two-year experiment was conducted at a commercial golf course in Charlottesville, VA to test suppression of plant-parasitic nematodes using biologically-based treatments. Treatments were i) *Steinernema feltiae* applied at a rate of 2.5 billion infective juveniles/ha, ii) *Photorhabdus luminescens* metabolites from liquid culture applied at rate of 9.35 L/ha, iii) Promax (a broad spectrum soil fungicide and nematicide with 3.5% thyme oil as the active ingredient) applied at rate of 9.35 L/ha, and iv) water (untreated control). In 2002, treatment with *S. feltiae* suppressed ($P \leq 0.05$) the population growth of *Mesocriconema* sp. one week after treatment. Treatment with *P. luminescens* suppressed ($P \leq 0.05$) the population growth of all plant parasitic nematodes except *Tylenchorhynchus* sp. one week after treatment and *Hoplolaimus* sp. six weeks after treatment. Treatment with Promax suppressed ($P \leq 0.05$) the population growth of *Mesocriconema* sp. and *Hoplolaimus* sp. six weeks after treatment. In 2003, treatment with *S. feltiae* suppressed ($P \leq 0.05$) the population growth of *Hoplolaimus* sp. one and six weeks after treatment. Treatment with *P. luminescens* suppressed ($P \leq 0.05$) the population growth of all plant parasitic nematodes expect *Tylenchorhynchus* sp. one and six weeks after treatment. Treatment with Promax suppressed ($P \leq 0.05$) the population growth of all plant parasitic nematodes one week after treatment and the population growth of *Hoplolaimus* sp. and *Helicotylenchus* sp. six weeks after treatment

BIOLOGICALLY-BASED MATERIALS AS POSTPLANT TREATMENTS FOR CONTROL OF NEMATODES ON ENGLISH BOXWOOD. **Perez, E. E., and E. E. Lewis.** Entomology Department, Virginia Polytechnic Institute & State University, Blacksburg, VA 24061.

A two-year experiment was conducted to test suppression of plant-parasitic nematodes on English boxwood using entomopathogenic nematodes and Promax (a broad spectrum soil fungicide and nematicide with 3.5% thyme oil as the

active ingredient). Treatments were *Steinernema riobrave* and *S. feltiae*, both applied at a rate of 2.5 billion infective juveniles/ha, and Promax at rate of 9.3 L/ha. In the 2001 season, treatment with *S. feltiae* reduced ($P \leq 0.05$) the population growth of *Tylenchorhynchus* sp. 7 days after treatment and *Hoplolaimus* sp. 30 and 60 days after treatment. Treatment with *S. riobrave* reduced ($P \leq 0.05$) the population growth of all plant-parasitic nematodes at all sampling dates, with the exception of *Mesocriconema* sp. 30 days after treatment and *Tylenchorhynchus* sp. and *Rotylenchus buxophilus* 60 days after treatment. Treatment with Promax reduced ($P \leq 0.05$) the population growth of all plant-parasitic nematode genera at all sampling dates except *Tylenchorhynchus* sp. and *R. buxophilus* 60 days after treatment. In the 2002, season treatment with *S. feltiae* had no effect on nematode population growth. Treatment with *S. riobrave* reduced ($P \leq 0.05$) the population growth of *R. buxophilus* 7 days after treatment, and all plant-parasitic nematodes 30 and 60 days after treatment except *Hoplolaimus* sp. 30 days after treatment and *Mesocriconema* sp. 60 days after treatment. Treatment with Promax reduced ($P \leq 0.05$) the population growth at all sampling dates of plant-parasitic nematodes except *Mesocriconema* sp. 60 days after treatment.

EFFICACY OF SELECTED BIOLOGICAL NEMATICIDES ON *PRATYLENCHUS PENETRANS* IN VITRO AND AFFECTING STRAWBERRY. **Pinkerton, J. N., and M. L. C. Kitner.** Horticultural Crops Research Laboratory, USDA-ARS, Corvallis, OR 97330.

Seven biological products reported to have nematcidal, nematistatic or plant-resistance-inducing properties were evaluated for the suppression of P. penetrans in vitro and on Totem strawberry plants. Mobility of nematodes was evaluated in vitro by exposing nematodes to 1200, 2400, or 4800 ppm of nematicide solutions for 24, 48, and 74 h, and 24 h after rinsing nematodes in deionized water. During the first 72 h, mobility was reduced 60–80% in Nemacur at 100 and 200 ppm, 28–80% in DiTera, and 28–58% in Dominator solutions. Most nematodes remained mobile in the other treatments (nematicidal castor oil, Promax, LCF, Sinosin). Except for Nemacur and DiTera at 4800 ppm, most nematodes recovered mobility following rinsing. Strawberry crowns were planted in pots containing ca. 2700 g of loam:sand (2:1) infested with 1.5 nematode g-1 soil. Nematicide solutions were applied at concentrations recommended by the manufacturers as drenches to soil field capacity. Applications were made at 28 day intervals starting at planting (preplant) or 30 day after planting (post-plant), except DiTera which was applied at 14 day intervals. Nemacur at 100 ppm, non-infested plants, and infested non-treated plants served as controls. Plants were maintained at 20–24 C with a 16 h photoperiod, and fertilized with each nematicide application. Plant growth and nematode population densities were assessed 16 weeks after the first nematicide application. Nemacur, Dominator, DiTera, and SLS suppressed nematode populations in the roots better than Promx, LCF, castor oil, and Sinosin. Biomass of roots and crowns were greatest with Nemacur, Dominator, DiTera, and Promax. In both studies, several products demonstrated potential for P. penetrans control, but none was as effective as Nemacur.

MERMITHID NEMATODES IN LARVAL MOSQUITOES: PASSIVE DISPERSAL. **Platzer, E. G., and J. Stephens.** Department of Nematology, University of California, Riverside, CA 92521.

Mermithid nematodes that specialize in infection of mosquito larvae face a dispersal problem since frequently adult mosquitoes are not infected. In this situation, several passive dispersal mechanisms can be invoked that include flooding and transport in mud on the body or feet of waterfowl. This process was tested for potential transport of postparasitic juveniles (J3's) of Romanomermis culicivorax. J3's were encapsulated in small pellets (ca 0.75 g) of moist kaolin and incubated at 17 °C for up to 24 h. At various intervals, pellets were dispersed in water and survival of J3's determined by observation of nematode motility. Motility was regained in 96% of the J3's after 24 hours in kaolin pellets. The ability of the recovered nematodes to mature and reproduce varied from 25 to 63% after 20 hours of encapsulation. Nematodes encapsulated for 24 hours did not produce eggs. Thus, J3's of R. culicivorax have the potential to survive mechanical transport for somewhat protracted periods in small amounts of pond mud that might be transported by waterfowl.

CHARACTERIZATION OF ROOT-KNOT NEMATODES RECOVERED FROM RICE-WHEAT FIELDS IN NEPAL. **Pokharel, R. R.,[1] G. S. Abawi,[1] J. M. Duxbury,[2] and C. Smart.[1]** [1]Department of Plant Pathology, NYSAES, Cornell University, Geneva, NY 14456; [2] Department of Crop and Soil Science, Cornell University, Ithaca, NY 14853.

Root-knot nematodes (*Meloidogyne* spp.) are a major constraint to rice and wheat production in South Asia. This study was carried out to determine the species and variability of *Meloidogyne* population occurring on rice and wheat in Nepal. Thirty-three samples of *Meloidogyne* were collected from different rice-wheat fields from the major production regions in Nepal and were maintained in the greenhouse on the rice variety BR 11. Morphometric measurements of second-stage juveniles, perennial patterns of mature females, virulence, and sequencing of the internally transcribed spacer (ITS) regions were determined for these samples. Morphometric measurements of juveniles indicated that the 33 root-knot samples examined were generally within the range previously reported for *M. graminicola*. The perennial patterns of the 33 root-knot samples were similar to that of *M. graminicola*, but there were some variability from the published pattern for this species. Amplification and sequencing of the ITS region exhibited a unique sequence that was not identical to any sequence for root-knot species available in the GeneBank. Symptoms of galls produced by the root-knot samples on rice

were similar to those reported for *M. graminicola*. The test of the virulence of the 33 root-knot samples on rice germplasm is on-going in the greenhouse.

REACTION OF COMMONLY GROWN RICE AND WHEAT VARIETIES IN NEPAL TO *MELOIDOGYNE GRAMINICOLA*. **Pokharel, R. R.,[1] G. S. Abawi,[1] J. M. Duxbury,[2] and C. Smart.[1]** [1] Department of Plant Pathology, NYSAES, Cornell University, Geneva, NY 14456; [2] Department of Crop and Soil Science, Cornell University, Ithaca, NY 14853.

Meloidogyne graminicola is an important pest of rice and wheat in South Asia. Ten rice and wheat varieties widely grown in Nepal were evaluated against the root-knot nematode (M. graminicola) in the greenhouse. Seeds were planted in clay pots filled with pasteurized soil infested with eggs of *M. graminicola* (5 eggs/cm^3 soil). The pots were maintained in the greenhouse at 25o C for 75 days. The roots were washed, rated for root galling severity (RGS) and eggs were then extracted from roots by the sodium hypo-chloride method. RGS was determined on a scale of 1 (no visible galls) to 9 (>80% roots of with galls). All the rice and wheat varieties tested were susceptible to *M. graminicola*, as determined by RGS ratings and nematode reproduction in roots. However, the wheat variety 'Brikuti', and the rice varieties 'Masuli', and 'Kanchi masuli' were the most susceptible. The wheat variety 'NL 297' and the rice varieties 'Mala' and 'Malasiya' were the most tolerant. The RGS of infected rice and wheat varieties ranged from 4.6–7.8 and 6.2–8.2, respectively. Similarly, the total number of larvae and eggs produced in roots ranged from 3808–32207 and 14324–32207/g root for the tested wheat and rice varieties, respectively. Further, screening of rice and wheat germplasm from several countries in South Asia is ongoing to identify sources of resistance in rice and wheat to *M. graminicola*.

PLANT-SOIL BIOTA FEEDBACKS AS A MECHANISM OF *MELALEUCA QUINQUENERVIA* INVASION IN THE FLORIDA EVERGLADES. **Porazinska, Dorota L.,[1] P. D. Pratt,[2] and R. M. Giblin-Davis.[1]** [1]Fort Lauderdale Research and Education Center, University of Florida, Fort Lauderdale, FL 33314; [2]Invasive Plant Research Laboratory, U. S. Department of Agriculture, Fort Lauderdale, FL 33314.

There are several leading theories explaining the extraordinary success of invasive plants but rarely are soil biota considered as an important factor mitigating plant invasions. We characterize plant-soil biota interactions in the form of a feedback system, where plant species affect the composition and abundance of soil biota (alteration of soil community) and the soil biota in turn feeds back to the plant community (alteration of aboveground diversity and productivity). We investigated the composition and diversity of nematode communities from soils dominated by the invasive tree *Melaleuca quinquenervia* as compared to adjacent soils supporting native non-invaded plant communities at 6 research sites in the Florida Everglades. Despite the significant geographical separation of the sites and their differences in soil type, hydrology, and native plant composition, there were consistent differences in nematode abundance and diversity between the tested plant communities. Abundances of bacterivores, herbivores, and total nematodes were suppressed in plant communities invaded by *M. quinquenervia* by 10, 75, and 45%. In addition, native stands were dominated by herbivores, while *M. quinquenervia* stands were dominated by bacterial feeders. The diversity of nematodes was also affected by the invasion process. Overall diversity of nematodes (genus level) was significantly lower (20%) under the exotic versus under the native plant communities. The decline of diversity was particularly high for herbivorous nematodes (50%). We also examined soil chemistry, texture, and plant diversity. The results provide strong support for the plant-soil biota feedback system as a mechanism of *M. quinquenervia* invasion of the Florida Everglades ecosystems.

MOLECULAR APPROACHES FOR DETECTING AND IDENTIFYING *PASTEURIA* SPECIES AND BIOTYPES. **Preston, J. F.,[1] L. M. Schmidt, [1] G. Nong,[1] D. W. Dickson,[2] J. A. Brito,[2] and R. M. Giblin-Davis.[2]** [1] University of Florida, Department of Microbiology and Cell Science, Gainesville, FL 32611-0700 ; [2] Entomology and Nematology Department, University of Florida, Gainesville, FL 32611.

Pasteuria spp. include those that are endospore-forming bacterial parasites of phytopathogenic nematodes. Propagation requires attachment of soil-borne endospores to juveniles of nematode hosts, infection, growth, sporulation, and release of endospores that repeat the cycle of infection and propagation. We have compared the sequences of selected sporulation genes and developed probes and primers that can be used to detect and distinguish different *Pasteuria* spp., including *P. penetrans* P20 from *Meloidogyne arenaria* race 1 and *Candidatus* Pasteuria usgae from *Belonolaimus longicaudatus*. The *sigE* genes have been particularly useful as targets for detection in infected plant tissues with PCR. Along with other sporulation genes, *sigE* probes and primers may be used to determine the extent to which nematodes infecting plants are themselves infected with *Pasteuria* early in the developmental process. This approach can complement the use of a monoclonal antibody to detect mature endospores as an estimate of the level of *Pasteuria* infection. Both approaches are useful for predicting the extent to which a soil plot may become suppressive for the development of root-knot and sting nematode, and in developing the potential of *Pasteuria* spp. for the biocontrol of plant-parasitic nematodes.

IDENTIFICATION OF ROOT-KNOT NEMATODES IN SOIL EXTRACTS BY PCR. **Qiu, Jack J., B. B. Westerdahl, C. Anderson, and V. M. Williamson.** Department of Nematology, University of California, One Shields Ave., Davis, CA 95616.

Molecular DNA approaches have been widely used in the taxonomy and identification of plant parasitic nematodes from pure cultures and hand-picked individuals, but not from soil extracts. We have developed a protocol for identification of the root knot nematode (RKN) species *Meloidogyne incognita, M. javanica* and *M. arenaria* in soil extracts using PCR. Nematodes are fractionated from soil using Baermann funnels and sucrose flotation. The nematode-containing fraction is then digested with proteinase K and a PCR assay is carried out with RKN-specific primers and with generic rDNA primers. RKN J2 can be detected among large numbers of other plant parasitic and free-living nematodes. The procedure works with a variety of soil types at various organic matter levels. It was found to have high sensitivity and accuracy in comparison with conventional microscopic assays for detection of RKN species.

EFFECT OF ROTATION CROPS, SOIL STORAGE TEMPERATURE AND INCUBATION PERIOD ON N-MINERALIZATION AND NEMATODES. **Quintanilla, M., S. Snapp, G. Bird, and J. Smith.** Departments of Entomology, Michigan State University, East Lansing, MI 48824.

The goal was to evaluate the impact of soil storage temperature and incubation period on soil nitrogen mineralization potential (SNMP) and nematode community structure associated with potato production systems. Three field experiments: a two-year bean-mustard trial, a six-year rotation trial with wheat, clover, rye and corn and a ten-year rotation with alfalfa were used. Potato-mustard resulted in significantly ($P = 0.05$) higher SNMP compared to a bean-mustard rotation, and SNMP was significantly higher ($P = 0.05$) following three days in cold storage and ten days of incubation, compared to other storage-incubation regimes. SNMP associated wheat-clover was significantly greater ($P = 0.05$) than that associated with rye, sweet corn or fallow soil. In addition, there were significant differences in SNMP associated with the length of incubation at 25 C, with the maximum occurring at 60 days. In the 10-year trial with continuous potato or 3-year alfalfa rotation, no differences in SNMP were observed after a year of corn. In the absence of incubation, there were no significant differences in population densities of bacterivores associated with continuous potato and the alfalfa rotation. Following 10 days of incubation after 40 days of storage at 10 C, population densities of bacterivores associated with continuous potato were significantly greater ($P = 0.05$) compared to those associated with the alfalfa rotation system. Incubation had no detectable impact on population densities of herbivores, fungivores, carnivores, omnivores, oligocheates or mycorrhizal fungal spores. In all experiments, soil storage temperature or incubation period significantly impacted SNMP.

STAN: A NOVEL APPROACH TO NEMATODE CONTROL IN COTTON. **Rideout, S. L.,[1] and D. H. Long.[2]** [1]Syngenta Crop Protection, Leland, MS 38756; [2]Syngenta Crop Protection, Greensboro, NC 27409.

Over the past two growing seasons, Syngenta has conducted trials in over 30 locations across the cotton belt examining the efficacy of a novel seed treatment nematicide. The use of a seed treatment (STAN-Seed Treatment Against Nematodes) for the control of nematodes in cotton offers many obvious benefits to producers, including, controlled application environment, precision seed to seed loading, reduced soil loading, and ease of use. Data from these trials indicates that STAN, reduced numbers of nematodes present in the soil and the plant, reduced galling, and increased plant vigor in areas infested with root-knot, Columbia lance, and reniform nematodes. Yields from STAN-treated plots were equal to or better than Temik when applied at 5 lbs/A. Additionally, no issues with phytotoxicity or crop safety were observed in STAN-treated plots. Further testing is planned by Syngenta with more emphasis being placed on early season plant and root samples in order to better understand the degree of nematode control offered by this unique product.

THE EFFECT OF VYDATE AND MOCAP ON *MELOIDOGYNE CHITWOODI*, OTHER PLANT- PARASITIC AND FREE-LIVING NEMATODES ON POTATOES IN WASHINGTON STATE. **Riga, E., and J. H. Wilson.** Washington State University, IAREC, Prosser, WA 99350.

Mocap and Vydate were evaluated for their effect on *Meloidogyne chitwoodi, Paratrichodorus allius, Pratylenchus penetrans,* and free-living nematodes on Russet Burbank potatoes. Mocap 6EC 12 Ibs + Temik 15G 3 Ibs significantly reduced *M. chitwoodi*. Free-living nematodes were not affected by any of the Mocap treatments except Mocap 6EC 12 Ibs + Temik 15G 3 Ibs. The following treatments significantly reduced the numbers of *P. penetrans:* Mocap 6EC 12 Ibs + Vapam HL 37.5 gal tank mix spring, Mocap 6EC 9 Ibs + Vapam HL 30 gal tank mix spring, Temik 15G 3 lbs banded and Mocap 6EC 12 Ibs + Temik 15G 3 Ibs at plant. Mocap 6EC 12 Ibs + Temik 15G 3 Ibs and Vydate CVL 2.I pt banded + 2.1 pints at 900 DDays significantly reduced P. allius. The percent infection, percent culls and infection index of the potato tubers by *M. chitwoodi* was significantly lower in all the treatments except with Mocap 6EC. Although all Vydate treatments significantly controlled *M. chitwoodi*, the following treatment provided the best control: Vapam 37.5 gal + Vydate 2.I pt banded + 2.1 pt at 900 DDays + 2.1 pt 14 days till end of season. Free-living nematodes were not affected by Vydate. Vydate treatments provided control against the *P. penetrans*, except Vydate 2.I pt + 2.1 pt 900 DDays. Vydate treatments provided control against *P. allius*. All Vydate treatments provided tuber protection against the root knot nematode. Although the yields were not significantly different in the Mocap and Vydate treatments,the chemical treatments were able to protect the potato tubers against the nematodes, especially against *M. chitwoodi*.

REPRODUCTION OF RENIFORM NEMATODE ON SOYBEAN, 2003 TESTS. **Robbins, R. T., L. Rakes,[1] L. E. Jackson,[1] E. E. Gbur,[2] and D. G. Dombek.[3]** [1] Department of Plant Pathology; [2]Agricultural Statistics Laboratory; [3]Akansas Crop Improvement Program, University of Arkansas, Fayetteville, AR 72701.

In 2003 greenhouse experiments,129 soybean varieties from the Arkansas variety testing program were tested in 10 cm-dia clay pots to determine their suitability as hosts for the reniform nematode, *Rotylenchulus reniformis*. The *R. reniformis* -resistant varieties Forrest and Hartwig, the susceptible variety Braxton, and fallow-*R. reniformis*-infested soil served as controls. Total number of eggs and nematodes extracted from both the soil and roots from each pot, reproductive indices (RI = Pf/Pi), RI/RI of Forrest (RF), RI/RI of Hartwig (RH), log ratio [log10 (RF + 1)], log ratio [log10 (RH + 1)], RF calculated from log10 (RF + 1), and RH calculated from log10 (RH + 1) were calculated for each cultivar or breeding line. Varieties with RF's significantly greater than the RF on Forrest (1.00) were considered suitable hosts for R. reniformis. In the 2003 Arkansas variety test only 7 of 129 lines did not have significantly more reproduction than Forrest when the log ratio [log10 (RF + 1)] were compared (Croplan Genetics RC4992, DT99-17145, Terral TVX57R301, Progeny 4884RR, Delta Grow 5650RR, FFR 4922RR, and Pioneer Brand 94M70). All seven cultivars had higher numerical ratios than Forrest. All lines including Forrest had more reproduction than Hartwig when the log ratio [log10 (RH + 1)] were compared.

DISTRIBUTION OF, AND INTRASPECIFIC VARIATION BETWEEN, POPULATIONS OF ENTOMOPATHOGENIC NEMATODES FROM BULL ISLAND, REPUBLIC OF IRELAND. **Rolston, A. N.,[1] S. Boyle,[2] T. Kakouli-Duarte,[2] C. T. Griffin,[1] and M. J. Downes.[1]** [1]Institute of Bioengineering and Agroecology, Department of Biology, National University of Ireland Maynooth; [2]Department of Applied Biology and Chemistry, Institute of Technology Carlow, Ireland.

Three species of entomopathogenic nematode (EPN) are present in Irish soils: *Steinernema feltiae*, *S. affine* and *Heterorhabditis downesi* (previously *Heterorhabditis* 'Irish type'). Over two years, a 100 × 800 m section of the dune system at Bull Island, Dublin Bay was surveyed for EPN by baiting soil cores (10 × 150 mm) with *Galleria mellonella* larvae. Soil cores were taken from a range of habitats from below the High Water Spring Tide strandline through to established dune grassland. Two species, *S. feltiae* and *H. downesi* were found, with each species having a prevalence of 2.8% (50/1760 cores) for the two sampling years combined. *Steinernema feltiae* was most prevalent towards the more stable grassland of the rear of the dune system. In contrast, *H. downesi* was most common 20 m into the dunes: an unstable area, the vegetation of which is dominated by marram grass (*Ammophila arenaria*. Molecular analysis of the beta-tubulin gene from Bull Island *S. feltiae* isolates and three *S. feltiae* out-groups, two from mainland Ireland (one from each of Co. Carlow and Co. Kildare) and one from England (UK76), was performed using Exon Primed, Intron Crossing (EPIC) PCR. The Bull Island isolates showed surprising variability given the small geographical distances between the isolates, suggesting multiple colonisation events of the island. Unintentional importation of soils that introduce new genetic lines of EPN is possible. Inadvertent local transfers of soil on the island itself may be important with regard to EPN dispersal and colonisation of new areas.

NOVAL MOLECULAR MARKERS FOR THE IDENTIFICATION OF THE SPECIES OF ENTOMOPATHOGENIC NEMATODE HETERORHABDITIS **Saeb, Amr T. M., and Parwinder S. Grewal.** Departement of Plant Pathology and Entomology, The Ohio State University, OARDC, Wooster, OH, 44691.

Entomopathogenic nematodes have tremendous potential for the control of many insect pests. However, identification and differentiation between the species of *Heterorhabditis* is very complicated task. This study aims to investigate potential use of noval molecular markers including the hear shock protein 70 gene(s), major sperm protein gene(s) and topoisomerase gene to differentiate between the species of Heterorhabditis. For the hsp70 gene family, four sets of primers were designed based on the free-living nematode *Caenorhabditis elegans* genomic sequences. One primer set targeted one exon area while the other three targeted the intron areas. The genomic DNA isolated from all 6 known *Heterorahbditis* species was subjected to polymerase chain reaction (PCR) using the primer sets. The amplified intron regions exhibited a distinct and reproducible pattern that characterizes different nematode species. The exon primer set was found to be specific for the species *H. megidis* for both the US and the European isolates. This exon amplified a product with a molecular weight of 307 bp. DNA sequencing data showed the amplification product is a pseudogene, *ϕ hsp70*. Both msp gene(s) and topoisomerase genes also showed distinct and reproducible patterns that characterized different *Heterorhabditis* species. This is the first study to use hsp70 gene family and topoisomerase gene as a target to differentiate between the entomopathogenic nematode species. The results of this study will facilitate rapid identification of the nematode species with simple PCR reactions and eliminate the need of inclusion of previously characterized nematode species in each test.

HORIZONTAL GENE TRANSFER IN *MELOIDOGYNE:* A COMPUTATIONAL APPROACH. **Scholl, Elizabeth H.,[1,2] J. L. Thorne,[2] J. P. McCarter,[3] and D. McK. Bird.[1,2]** [1]Center for the Biology of Nematode Parasitism; [2]Bioinformatics Research Center, North Carolina State University, Raleigh, NC 27695; [3]Divergence Inc., St. Louis, MO 63141.

Horizontally transferred genes in plant-parasitic nematodes have traditionally been identified by characterization of

individual genes *via* biochemical and immunological criteria rather than as a result of a specific search for gene transfer *per se*. We have developed a comprehensive two-step comparative genomic approach using a phylogenetic filter to identify horizontal gene transfer candidates in three species of *Meloidogyne*. This approach identified previously postulated horizontally transferred genes and revealed six new candidates. Computational and experimental methods verified the horizontal gene transfer candidates as *bona fide* nematode genes. Phylogenetic analysis implicated rhizobial ancestors as donors of horizontally acquired genes in *Meloidogyne* and suggests a link between horizontally transferred genes in *Meloidogyne* and parasitism.

DENSITY-DEPENDENT EFFECTS ON HATCH IN THE SOYBEAN CYST NEMATODE. **Schroeder, N. E., and A. E. MacGuidwin.** Department of Plant Pathology, University of Wisconsin Madison,WI 53706.

Hatching stimulants from cyst fragments and egg homogenate of the soybean cyst nematode have been reported. We propose that these stimulants create a density dependent effect on hatch. Hatching studies were undertaken to determine whether total cyst population density affects hatch. Hatching chambers, 2-cm in diameter, were fitted with 25-μm nylon screens and filled with 2 ml de-ionized water. Cysts of similar size and appearance were extracted from 3-month old pot cultures, surface sterilized with 0.5% NaOCl for 1 minute and placed on the screens in a randomized design. Treatment levels of 1,2,4,8 or 16 cysts were used with 13 replications per treatment. Chambers were stored together in a plastic box and placed in a dark, 25 °C incubator. The hatching chambers were emptied, refilled with fresh water, and the juveniles counted at four sampling times over a 19-day period. The data were expressed as hatched juveniles per cyst for each sampling date. All data were log(x + 1) transformed. The hatch per cyst was greater ($P < 0.05$) for the 16-cyst treatment than the 1-cyst treatment for the first 3 sampling dates. Hatch per cyst was greater ($P < 0.05$) for the 8-cyst treatment than the 1-cyst treatment for the first 2 sampling dates.

PRELIMINARY OBSERVATIONS ON THE SYSTEMATICS OF THE MARINE NEMATODE FAMILY, COMESOMATIDAE, BASED ON MOLECULAR DATA. **Sharma, Jyotsna,[1] L. Sun,[2] D. Hope,[3] and V. R. Ferris.[2]** [1]Department of Biology, University of Texas, San Antonio, TX 78249; [2]Department of Entomology, Purdue University, West Lafayette, IN 47907; [3]Smithsonian Institution, NMNH, Washington, DC 20560.

Free-living nematodes are a major component of marine benthic habitats both in abundance and diversity, and are important as primary and secondary consumers in benthic food chains. In ecological collections from benthic areas of the Texas Gulf, species of Comesomatidae have comprised up to 40% of the nematodes recovered. The higher phylogenetic relationships of the comesomatids remain unresolved, owing to the fact that they have diagnostic morphological characteristics of both the Monhysterida (outstretched female gonoducts) and of the Chromadorida (multispiral amphids). We are involved in a long-term study to assess relationships of the comesomatids, based on additional morphological characters, and also on molecular data. All specimens for our molecular study have been collected from The Aransas Pass, Port Aransas, Texas, USA. We now have partial (660 base pairs) sequence data from the ribosomal RNA 18S gene (18S rDNA) for marine nematodes, including two comesomatid species, *Dorylaimopsis metatypicus* and *Sabatieria sp*. We also have new data for *Terschellingia longicaudata*, a monhysterid, and for a chromadorid species, *Chromadorita pharata*. We analyzed these data, along with GenBank data from the same area of 18S rDNA for additional marine nematode species, *viz*., *Stilbonema sp*. and *Desmodora sp*. (both chromadorids) and for *Plectus sp*. and *Enoplus sp*. In our analyses the two species of Comesomatidae grouped with the monhysterid and not with the chromadorids. We plan to collect and analyze molecular data from additional taxa of these and other families of free-living nematodes.

CONTROL OF *RADOPHOLUS SIMILIS* IN *ANUTHURIUM ANDRAEANUM* WITH AVID. **Sipes, B. S., K. Sewake, and B. Chinnasri.** Department of Plant and Environmental Protection Sciences, University of Hawaii, 3190 Maile Way, Honolulu, HI 96822.

Two experiments were conducted to determine the efficacy of the avermectin Avid against *Radopholus similis* in *Anthurium andraeanum*. In the first experiment, tissue cultured plants growing in 10-cm-square pots filled with volcanic cinder were inoculated with 0 or 1000 *R. similis* plant. Twelve weeks after inoculation, the pots were drenched with 250 ml of a 0 or 1.3 ml Avid 0.15 EC/l water solution. The pots were drenched again 7, 14, and 21 days later. Each treatment was replicated 10 times and arranged in completely randomized blocks on greenhouse benches. Two weeks after the last treatment the plants were harvested. Shoot and root wet weights were recorded. Roots and stems were separately cut into 1-2 cm pieces and a 20 g subsample of each placed into a mist extractor for 3 days to collect nematodes. The uninoculated plants were larger than the inoculated plants (160 vs 113 g). The avermectin treatment reduced nematode numbers in the roots from 266 to 3 and nematode numbers in the stems from 6 to 0. In the second experiment, control was evaluated with Avid 0.15 EC at 0, 0.1, 0.3, 0.7, and 1.3 ml/l water in 150 ml drenches. Methods were similar to the first experiment except that the four drenches were 14 days apart. Plant weights were similar regardless of the Avid rate. Nematode populations decreased with increasing concentrations of Avid (284, 126, 9, 3, and 2 nematodes/20 g root and16, 23, 31, 0, and 0

nematodes/20g stem at 0, 0.1, 0.3, 0.7, and 1.3 ml Avid/l water respectively). Avermectins provide exceptional control of *R. similis* in cinder-grown anthurium.

MORPHOLOGICAL AND MOLECULAR EVALUATION OF A *MELOIDOGYNE HAPLA* POPULATION DAMAGING COFFEE (*COFFEA ARABICA*) IN MAUI, HAWAII. **Skantar, Andrea M.,[1] Handoo, Z. A.,[1] Carta, L. K.,[1] and Schmitt, D. P.[2]** [1]USDA ARS Nematology Laboratory, Beltsville, MD 20705; [2]Department of Plant and Environmental Sciences, University of Hawaii at Manoa, Honolulu, HI 96822.

Here we describe an unusual population of *Meloidogyne hapla* that causes significant damage to coffee on Maui, Hawaii. Earlier thought to be an undescribed species, this population represents the first report of *M. hapla* on coffee beyond that previously reported from Brazil. The Maui population caused large galls devoid of proliferating rootlets. Identification was verified by morphology, esterase isozyme pattern, and DNA sequences. Twenty percent of perineal patterns had perpendicular lines atypical for the species. Female interphasmidial distance was relatively high, and anus to tail terminal distance was intermediate among five populations. The average length of juveniles from this Hawaiian population was considerably smaller than for most other populations of *M. hapla*. Populations sampled in summer and winter had significantly different juvenile body lengths. Tail lengths were different from type populations but similar to an atypical African population. We examined five DNA markers, including the 28S ribosomal large subunit (LSU-D3), internal transcribed spacer 1 (ITS1), intergenic spacer (IGS), the mitochondrial interval spanning from cytochrome oxidase II to 16S, and the nuclear gene Hsp90. Sequences for ITS1, IGS and mitochondrial genes were similar to other populations of *M. hapla*, and LSU-D3 revealed the presence of two haplotypes similar to sequences reported previously. Hsp90 DNA sequences from Hawaii and Maryland populations differed at only two to five nucleotides, but varied significantly from Hsp90 in other *Meloidogyne* species. Molecular and morphological features are discussed along with host range, cultivar, and biogeographical issues.

EARLY PROTECTION OF PIMA COTTON SEEDLINGS BY ABAMECTIN SEED COATING AGAINST ROOT-KNOT NEMATODES. **Smith Becker, J., and J. O. Becker.** Department of Nematology, University of California, Riverside, CA 92521.

Early development and function of the root system of cotton (*Gossypium hirsutum*) is critical to the growth of the whole plant and its yield potential. Under optimal conditions, the primary root penetrates the soil rapidly and may reach a depth of 20 cm by the time the cotyledons emerge from the soil. Lateral roots grow outward, forming a mat of roots that are critical for seedling vigor. Early attack by root-knot nematodes can stunt root growth that is difficult to overcome. In 6-wk greenhouse trials at 25C, non-treated and abamectin-coated (0.1 mg a.i./seed) cotton cv. Pima S-7 were challenged in steam-pasteurized sandy soil with *Meloidogyne incognita* race 3 at initial population densities ranging from 0–1,000 J2/100cm^3 soil. Differences in seedling height became apparent after approximately 3 weeks. As few as 50 J2/100cm^3 caused significant reduction in total root length at trial termination. Root length reduction increased proportionally with increasing initial root-knot nematode population densities. Seed coating with abamectin resulted in root and shoot length similar to the non-infested control regardless of the infestation level.

DYNAMICS OF SUGAR BEET CYST NEMATODE POPULATIONS IN SUGAR BEET PRODUCTION FIELDS IN WASHAKIE COUNTY, WYOMING. **Smith, H. J., F. A. Gray, and D. W. Koch.** Department of Plant Sciences, University of Wyoming, Laramie, WY 82071.

Site-specific management of sugar beet cyst nematode (*Heterodera schachtii*) (SBN) is feasible if population dynamics are known and additional cost is economically justified. The objective of this work was to investigate the variation in spatial and temporal distribution of SBN in four sugar beet production fields in Washakie County, Wyoming and to determine how often SBN sampling is required. Fields had been in a continuous sugar beet – malting barley rotation for many years. Two fields (12 samples each with grid sizes of 0.13 ha and 0.25 ha) were sampled in the fall of 1999, 2001, and 2003 after barley harvest. Two fields (15 and 18 samples each with grid sizes of 0.16 ha and 0.21 ha) were sampled in the spring of 2001 and 2003 before growing sugar beets. SBN population densities were determined in eggs+J2/cm^3 soil. The spatial distribution of SBN was mapped by inverse distance weighting. Population densities among years were compared with paired t-tests. Initial average SBN density in 1999 was 12.8 eggs+J2/cm^3 for field 1 and 11.3 eggs+J2/cm^3 for field 2. There were no changes in SBN densities from 1999 to 2001 ($P > 0.05$). However, populations were significantly lower in 2003 than in the previous years for both fields ($P < 0.05$). Initial average SBN density in 2001 was 1.6 eggs+J2/cm^3 for field 3 and 3.5 eggs+J2/cm^3 for field 4. SBN densities from 2001 to 2003 in both fields did not change ($P > 0.05$). Results show that fields need to be sampled at least every two years before growing sugar beet.

PARASITISM OF *TRICHODERMA* ON ROOT-KNOT NEMATODES – MECHANISMS AND IMPROVED BIOCONTROL. **Spiegel, Yitzhak,[1] I. Chet,[2] A. Herrera-Estrella,[3] and E. Sharon.[1]** [1]Division of Nematology, ARO, Volcani Center, Bet Dagan 50250, Israel; [2]Department of Biological Chemistry, Weizmann Institute of Science, Israel;[3]Centro de

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The fungus Trichoderma harzianum (asperellum) exhibits biocontrol activity against the root-knot nematode Meloidogyne javanica. Direct fungal parasitism is one of various possible mechanisms by which the fungus can act against the nematode juveniles (J2) and eggs. The fungal hyphae coil around the nematode eggs and J2 before penetrating them. A constitutive GFP(green fluorescent protein)-transformant of T-203 was used to observe the fungal parasitism on nematode eggs and juveniles, using laser scanning confocal microscopy. The involvement of lytic enzymes in the process was demonstrated using inductive GFP-fungal transformants. A transgenic strain, carrying a fusion of the proteinase prb1 promoter with the gfp gene, was used to demonstrate that this gene is turned on during the interaction between the fungus and the nematode. Activities of chitinolytic enzymes were also induced in the presence of nematode eggs. T-203 gfp constracts with the promoters of endochitinase gene chit36 and the N-acetyl-glucoseaminidase gene chit102 were used to visualize these activities. The direct fungal parasitism process on the nematode could be improved, in vitro, by using antibodies. Antibodies against root-knot nematodes, that bound to the surface of M. javanica J2 and eggs, bound also to the fungal spores and agglutinated them. The presence of these antibodies enhanced the attachment of the spores to the nematodes. This resulted in a significant enhancement of fungal parasitism on the nematodes. Moreover, the binding of antibodies to the spores enhanced their germination and thus improved the fungal parasitism on the nematodes.

THE EFFECTS OF SODIUM CHLORIDE ON EMBRYONIC DEVELOPMENT AND EGG HATCH OF THE SOUTHERN ROOT-KNOT NEMATODE, *MELOIDOGYNE INCOGNITA*. **Stoner, N., K. Nagle, M. Rivera, M. Schroeder, and L.VanSant.** DuPont Crop Protection, Stine-Haskell Research Center, Newark DE 19714.

Eclosion of infective second stage larvae can be highly synchronized when eggs at various stages of embryonic development are incubated in 0.3M NaCl for up to 3 weeks prior to transferring to deionized water. Cumulative hatch recorded up to 7 days after egg harvest showed a 2.7 fold increase in J2 production over the control group incubated in deionized water. Further, the eggs held in the saline solution completed hatching within 48 hours. This technique offers several advantages to researchers seeking to standardize laboratory bioassays.

PHYLOGENY OF THE SUBORDER CRICONEMATINA: VIEW FROM THE ANALYSIS OF THE D2-D3 EXPANSION REGIONS OF THE 28S RDNA. **Subbotin, S. A.,[1] N. Vovlas,[2] R. Crozzoli,[3] D. Sturhan,[4] F. Lamberti,[2] M. Moens,[5] and J. G. Baldwin.[1]** [1]Department of Nematology, University of California, Riverside, CA 92521; [2]Istituto per la Protezione delle Piante, Sezione di Bari, C.N.R., 70126 Bari, Italy; [3]Instituto de Zoologia Agricola, Universidad Central de Venezuela, Apdo. 4579, Maracay, Venezuela; [4]Institut fur Nematologie und Wirbeltierkunde, Munster, Germany; [5]CLO-Department for Crop Protection, 9820, Merelbeke, Belgium.

The suborder Criconematina Siddiqi, 1980 includes a large group of ecto- and endoparasitic nematodes with several, including *Tylenchulus semipenetrans* and *Mesocriconema xenoplax*, being of major agricultural importance. The D2-D3 expansion segments of the 28S nuclear ribosomal DNA were amplified and sequenced from 36 populations of species from the genera *Mesocriconema, Ogma, Criconema, Hemicycliophora, Hemicriconemoides, Xenocriconemella, Criconemoides, Nothocriconema, Trophonema, Tylenchulus, Sphaeronema, Paratylenchus* and two outgroup taxa from Tylenchidae (*Coslenchus* and *Aglenchus*). Alignment was optimized using the secondary structure model, and was analyzed using maximum parsimony, maximum likelihood and Bayesian interference approaches. The three analyses produced trees with similar topology. Although the molecular trees differ from the previous morphological-based hypotheses of criconematid phylogeny proposed by Siddiqi (2000) and Maggenti *et al.* (1987), maximum likelihood tests did not yield statistically significant differences between some of the tested classical and molecular topologies. DNA data supports monophyly for genera *Mesocriconema* and *Hemicriconemoides* and rejects the hypothesis of a single origin of criconematids with a double cuticle. Substantial sequence divergence in this DNA segment between populations of *Mesocriconema sphaerocephala* or *Hemicriconemoides cocophillus* may suggest the presence of several sibling species under these taxon names.

A SYNOPSIS OF THE "POTPOURRI OF NEMATOLOGICAL METHODS AND TECHNIQUES." **Tarjan, Armen Charles.** Professor Emeritus, Department of Entomology & Nematology, University of Florida, Gainesville. FL 32611.

Before any feat, nematological or otherwise. can be accomplished, a method of execution must be at hand. As is often done, the investigator relies on his own ingenuity to devise the needed technique. Lamentably, however, such innovations later are entombed in the methods section of the manuscript and often forgotten. Thus it becomes clear that an effort should be made to resuscitate such forgotten techniques, which the present ongoing investigation purports to do. This work consists of methods dating back to the 19th century, but primarily from the mid to late 20th century. At present, there are almost 1100 entries, each a 2- to 4-sentence long abstract of published research. The file embraces well over 100 nematological subject categories. At present, it is estimated that the work is 75 to 80% complete. The file can be accessed on the World Wide Web at:—http://methodsfile.ifas.ufl.edu—.

GENOMICS REVEALS KEY TRANSPORT PROCESSES ACROSS MEMBRANES OF NEMATODE-INDUCED GIANT CELLS. **Taylor, Christopher G., Ulrich Hammes, Erik Nielsen, and Daniel Schachtman.** Donald Danforth Plant Science Center, St. Louis, MO.

Root-knot nematodes (*Meloidogyne* spp.) have evolved a very sophisticated mechanism to feed from plants. Upon entry to roots the root-knot nematode selects several plant cells, which are induced to grow into giant cells. These giant cells are 50-100 times larger than surrounding plant cells and have many of the hallmarks of transfer cells including: dense cytoplasm; numerous small vacuoles; thickened and invaginated cell walls; large numbers of mitochondria and increased metabolic and transport activity. Because the giant cell plasma membrane is highly invaginated with few plasmadesmotal connections to the surrounding cells, membrane transporters in the plasma membrane will play an important role in the uptake of specific nutrients require by the nematode for growth and development. To identify the key transporters in the giant cell membranes we have initially used a genomic approach. Using the Affymetrix GeneChip *Arabidopsis* ATH1 Genome Array we identified at least 57 genes encoding membrane transport proteins that were consistently up-regulated at 1, 2, or 4 weeks after nematode infection. The results were verified using real time PCR. The expressions of the genes were found to fall into three different categories according to where they were expressed (entire root, feeding sites, and the root outside feeding sites). The results from the analysis of the whole genome expression data will be presented with emphasis on the expression of genes encoding transport proteins.

SOIL NEMATODE COMMUNITIES IN A CONTINENTAL CLIMATE WITH COLD WINTERS RESPOND TO AGRICULTURAL MANAGEMENT. **Tenuta, Mario.** Department of Soil Science, University of Manitoba, Winnipeg, Manitoba, Canada R3R 2N2.

The response of soil nematode communities to agricultural management practices in Manitoba Canada (continental moist mid-latitude climate with cold winters) was assessed. In summer 2003 three sites from southern Manitoba covering a range of management intensities were sampled: a loamy sand having impoverished prairie, pasture prairie, and potato-cereal fields; a clay soil in its 13th year as restored prairie (previously in cereal cultivation) and 13th year flax phase of three, four-year rotations managed conventionally or organically (without fertilizer and herbicide); a silt loam soil in its 6th year potato phase of five rotations. Nematode trophic groups indicated soil food webs of the restored and pastured prairie to be dominated by herbivores *(Tylenchus, Helicotylenchus*) and bacterivores (*Plectus, Acrobeles*), potato soil by bacterivores (Rhabditidae) and fungivores (*Aphelenchus*), and the flax soil by bacterivores (*Eucephalobus*), herbivores (*Filenchus*) and fungivores (*Aphelenchus*). Poor plant productivity of the impoverished prairie was reflected in low total nematode counts and reduced presence of herbivores. Other taxa were associated with particular management, *Helicotylenchu*s where wheat grown and *Xiphinema* in potato-wheat-canola-wheat rotation. Thirteen years of management as restored prairie altered dominance of the nematode community to several taxa of the Tylenchidae and to Eudorylaimus. The Nematode Enrichment Index values of the pasture prairie and impoverished prairie were lower than all other soils (about 0.50 compared to >0.70). The Nematode Structure Index values of soils decreased with management intensity ranging from about 0.75 for the prairie soils (lowest management intensity) to 0.15 for potato soils (highest management intensity). This trend was also evident for management intensity of the flax rotation, Nematode Structure Index values were higher for organic (0.48) than conventional (0.35) management. The results indicate that for continental climates with cold winters analysis of nematode communities can be a valuable means of describing the effects of management on the structure of soil food webs and their functioning.

STABILITY OF RESISTANCE TO ROOT-KNOT NEMATODES IN BELL PEPPERS IN A SUB-TROPICAL ENVIRONMENT. **Thies, Judy,[1] D. W. Dickson,[2] and R. L. Fery.[1]** U.S. Vegetable Laboratory, USDA, ARS, Charleston, SC 29414; [2]Department of Entomology and Nematology, University of Florida, Gainesville, FL 32611.

Two root-knot nematode resistant bell pepper cultivars 'Charleston Belle' and 'Carolina Wonder' (*Capsicum annuum* L. var. *annuum*) and their susceptible recurrent parents, 'Keystone Resistant Giant' and 'Yolo Wonder B', were compared for managing the southern root-knot nematode (*Meloidogyne incognita*) in fall and spring trials at Gainesville, FL. In the fall trial, 'Charleston Belle' and 'Carolina Wonder' exhibited minimal root galling and nematode reproduction, and 'Keystone Resistant Giant' and 'Yolo Wonder B' exhibited severe root galling and high nematode reproduction. Fruit yields of 'Charleston Belle' were 49% greater than yields of the two susceptible cultivars ($P < 0.006$). In the spring trial, one-half of the plots were treated with methyl bromide before planting the same four bell pepper cultivars. 'Keystone Resistant Giant' and 'Yolo Wonder B' grown in untreated control plots exhibited severe root galling and high nematode reproduction, but the other six cultivar × methyl bromide combinations exhibited minimal root galling and nematode reproduction. These results demonstrate that root-knot nematode resistant cultivars such as 'Charleston Belle' and 'Carolina Wonder' are a viable alternative to methyl bromide for managing southern root-knot nematode in bell pepper in sub-tropical environments.

THE EFFECT OF PREVIOUS HOST ON SUBSEQUENT VIRULENCE OF *MELOIDOGYNE INCOGNITA* ON CHILE PEPPER AND NUTSEDGES. **Thomas, S. H.,[1] J. M. Fuchs,[1] J. Schroeder,[1] and L. W. Murray.[2]** [1]Department of

Entomology, Plant Pathology, and Weed Science, and [2]University Statistics Center, New Mexico State University, Las Cruces, NM 88003.

The purpose of greenhouse studies conducted in 2001 and 2002 was to determine if previous host or crop/weed competition affect the fitness of *Meloidogyne incognita* on subsequent hosts. The following combinations of chile pepper (*Capsicum annuum*), yellow nutsedge (*Cyperus esculentus* = YNS), and purple nutsedge (*C. rotundus* = PNS) were tested: chile, YNS, and PNS alone, chile+YNS, and chile+PNS. Pots were inoculated with 5,000 *M. incognita* eggs recovered from chile, YNS, or PNS and harvested 45 days later. Tomato (*Lycopersicum esculentum*) was included as an inoculated control. Cumulative egg hatch was determined for each inoculum source and used to adjust differences in egg viability at inoculation. At harvest, eggs were extracted from half of each root system, and the remaining half was stained with Phloxine B for assessment of egg mass production and numbers of eggs per egg mass. The level of nematode reproduction, as indicated by the number of eggs and egg masses produced per plant or per gram of root, differed among inoculum sources. For example, reproduction on nutsedges was less with inoculum produced on chile than with inoculum from YNS or PNS. However, the number of eggs produced per egg mass was unaffected by inoculum source or plant competition. Except for reductions in nutsedge root growth by *M. incognita* inoculum from PNS, no differences in pathogenicity occurred among inoculum sources for any host or host combination. These findings indicate that *M. incognita* progeny from different hosts may differ in their ability to infect subsequent hosts.

MOLECULAR PHYLOGENY OF *FERGUSOBIA* SPECIES (TYLENCHIDA: FERGUSOBIINAE) INFERRED FROM NUCLEAR RIBOSOMAL AND MITOCHONDRIAL DNA SEQUENCE DATA. **Thomas , W. K.,[1] Ye, W. M.,[2] R. M. Giblin-Davis,[2] K. A. Davies,[3] M. F. Purcell,[4] S. J. Scheffer,[5] G. S. Taylor,[3] T. D. Center,[6] and K. Morris.[1]** [1]Hubbard Center for Genome Studies, University of New Hampshire, Durham, NH 03824; [2] University of Florida/IFAS, 3205 College Ave., Davie, FL 33314; [3] University of Adelaide, South Australia 5064, Australia; [4] USDA Australian Biological Control Laboratory, Queensland 4068, Australia; [5] USDA-Agricultural Research Service, Systematic Entomology Lab, Beltsville, MD 20705; [6]USDA-Agricultural Research Service, Invasive Plant Research Lab, 3205 College Ave., Davie, FL 33314.

DNA sequences of the nuclear ribosomal DNA near-full length small subunit (1685−1688 bp), partial large subunit D2/D3 domain (850−899 bp), and partial mitochondrial cytochrome oxidase subunit I (618bp) were analyzed to construct the evolutionary relationships of *Fergusobia* species. The analysis included 99 isolates of *Fergusobia* species from a variety of myrtaceous hosts in Australia. Phylogenetic analysis using neighbor joining and maximum parsimony inference based on the 3 loci were congruent with notable exceptions. The resultant SSU phylogeny supported a monophyletic *Fergusobia* genus, with *Howardula* as the outgroup. Sequence analysis revealed a large number of monophyletic clades within *Fergusobia* species. Those clades are generally consistent with morphological data, fly host species and plant host species. In many cases, phylogenetic analysis revealed the existence of cryptic species and host switching events. Sequence data of SSU, LSU and mtCOI supply sufficient phylogenetic information across this broadly divergent genus. Among the 3 loci, LSU was the most informative across this genus and mtCOI was limited by apparent saturation at synonymous coding positions.

HOST STATUS OF PEARL MILLET FOR STING, STUBBY-ROOT, AND LESION NEMATODES. **Timper, P.,[1] and W. W. Hanna.[2]** [1]USDA ARS, Crop Protection and Management Research Unit, P.O. Box 748, Tifton, GA 31793; [2]Department of Crop and Soil Sciences, University of Georgia, P.O. Box 748, Tifton, GA 31793.

New hybrids of pearl millet (*Pennisetum glaucum*) have recently been developed for use as a grain crop in the southern United States. Because this crop is extremely drought tolerant and resistant to mycotoxins, it has tremendous promise as an alternative feed grain for dryland production. The pearl millet hybrid TifGrain 102 is resistant to both *Meloidogyne incognita* race 3 and *M. arenaria* race 1; however, its host status for other important plant-parasitic nematodes was unknown. The objective of this study was to determine reproduction of *Belonolaimus longicaudatus*, *Paratrichodorus minor*, and *Pratylenchus brachyurus* on two pearl millet hybrids (HGM-100 and TifGrain 102) compared to reproduction on cotton (*Gossypium hirsutum*), corn (*Zea mays*), and peanut (*Arachis hypogaea*). Each nematode species was tested in separate greenhouse experiments with seven to nine replicate pots per crop. Two months after inoculation, sting and stubby-root nematodes were extracted from soil by centrifugal flotation, and lesion nematodes were extracted from roots by Baermann funnel/mist chamber. Each experiment was conducted twice. The relative host status of the crops for the nematodes is as follows. Peanut and the two millet hybrids were poor hosts for *B. longicaudatus*, whereas cotton and corn were good hosts. Peanut and TifGrain 102 were poor hosts for *P. minor*, whereas cotton, corn, and HGM-100 were good hosts. Both millet hybrids were poor hosts for *P. brachyurus*, whereas cotton, corn, and peanut were good hosts. Growing TifGrain 102 in rotation with other crops should not lead to damaging populations of sting, stubby-root, or lesion nematodes.

SENTINEL NEMATODES OF LAND USE CHANGE AND RESTORATION. **Todd, T. C.,[1] T. O. Powers,[2] and P. Mullin.[2]** [1] Department of Plant Pathology, Kansas State University, Manhattan, KS 66506; [2]Department of Plant Pathology, University of Nebraska, Lincoln, NE 68583.

Changes in land use and the associated changes in land cover are recognized as the most important component of human-induced global change. Much attention has been focused on deforestation, but grasslands are among the most endangered ecosystems on Earth. The North American tallgrass prairie is a dramatic example, exhibiting a greater than 95% decline in historical area. Renewed interest in prairie conservation and restoration has elucidated the need for ecological indicators of disturbance and recovery in native systems, including those associated with critical soil processes. The tallgrass prairie differs from the agricultural systems that replaced it in having greater diversity and heterogeneity of resources, less physical soil disturbance (although other disturbances, such as fire and grazing, are prominent), and greater nitrogen limitation. Understanding the responses of nematode taxa to these characteristic differences is crucial to the development and improvement of community indices, but while knowledge of disturbance responses by individual taxa is accumulating, the level of necessary taxonomic resolution remains in question. Although nematode communities generally are better described for temperate grasslands than for other natural ecosystems, identification of sentinal taxa is confounded by high levels of diversity, and both spatial and temporal heterogeneity.

MEMBRANE ARRAY AND RT-PCR ANALYSIS OF TWO SUBTRACTION CDNA LIBRARIES FOR NEMATODE-INFECTED SOYBEAN ROOTS. **Tucker, M. L., A. Raina, V. K. Thai, and P. Xue.** Soybean Genomics and Improvement Lab, Agricultural Research Service, U. S. Department of Agriculture, Beltsville, MD, 20704.

Soybean cyst nematode (SCN) is the most economically destructive pathogen of soybeans. Identification of gene expression that is upregulated during infection by SCN and also highly specific to the infection site will provide additional tools to engineer SCN resistance in soybean. We have prepared two subtraction libraries to enrich for SCN-induced genes expressed in the early and late stages of the 30-day lifecycle of SCN in soybean roots. In addition to subtraction with cDNA prepared from uninoculated roots, cDNA from SCN eggs were also used for subtraction. Two thousand cDNA clones were selected from each library and stored in 96-well microtiter plates. From each of these libraries 384 cDNAs were sequenced from both directions and the cDNAs arrayed onto nitrocellulose membranes. The membrane arrays were then hybridized to radioactively labeled cDNAs prepared from total root RNA isolated at 4, 12, and 20 days post-SCN-inoculation (PSI) and RNA from 0, 4, 12 and 20 days incubation with no inoculation. The 384 cDNAs from the early subtraction library (1, 2 and 4 days PSI) do not include any SCN transcripts and no host genes that were significantly upregulated at our conditions. However, 190 of the 384 cDNAs from the late subtraction library (8, 12, 20 days PSI) have high sequence identity with nematode sequences and most were associated with GenBank SCN ESTs from maturing nematodes. Moreover, a large number of the soybean cDNAs that were upregulated by the SCN infection in the late library were associated with previously identified nodulation (nod) genes. In addition to nod genes, there were a few upregulated cDNAs that were not previously reported to be associated with SCN infection and have been examined further by RT-PCR.

SCN SOYBEAN YIELD-LOSS ESTIMATES – FACTORS TO CONSIDER IN AN IDEAL WORLD. **Tylka, G. L.** Department of Plant Pathology, Iowa State University, Ames, IA 50011.

Accurate and meaningful estimates of reductions in crop yields due to damage caused by plant-parasitic nematodes are difficult to determine for individual fields; doing so for entire states is orders of magnitude more challenging. But state- and region-wide yield-loss estimates are requested frequently by media and are needed by companies and granting agencies to make research funding decisions. Developing estimates of damage caused to soybean, *Glycine max*, by the soybean cyst nematode (SCN), *Heterodera glycines*, is particularly challenging in the Midwestern United States because of the vast area of land on which the crop is grown, the absence of symptoms of damage in many *H. glycines*-infested fields, and the lack of information on the distribution, densities, and virulence of the *H. glycines* populations in the various states. Ideally, yield-loss estimates would account for the distribution, density, and relative virulence of *H. glycines* populations as well as the effects of adverse edaphic conditions, such as improper soil fertility and inadequate moisture levels, on yield reductions caused by the nematode. Also, reductions in soybean yield caused by *H. glycines* through interactions with other plant-parasitic nematodes, other plant pathogens, and other pests, such as insects and weeds, ideally would be included in calculations of yield-loss estimates as well. Finally, yield-loss estimates ideally would include consideration of the tolerance and resistance of soybean varieties grown in an area, as these varietal traits would affect the magnitude of damage caused by *H. glycines*. It may never be possible to account for all of the aforementioned variables when calculating *H. glycines*-induced yield-loss estimates, but a method that considers the effects of at least some of the variables in a standardized manner is needed.

COTTON VARIETY RESPONSE TO *ROTYLENCHULUS RENIFORMIS* IN ALABAMA. **Usery Jr., S. R.,[1] K. S. Lawrence,[1] C. H. Burmester,[2] K. Glass,[2] R. Akridge,[2] B. A. Meyer,[3] and G. W. Lawrence.[4]** [1] Departments of Entomology and Plant Pathology, [2] Department of Agronomy and Soils, Auburn University, Auburn, AL 36849; [3] Delta and Pine Land Co., Hartselle, AL, 35640; [4]Department of Entomology and Plant Pathology, Mississippi State University, Mississippi State, MS, 39762.

Currently management of the reniform nematode *Rotylenchulus reniformis* is limited to crop rotations and nematicides. Selected cotton varieties were screened for resistance in the greenhouse and for tolerance in field locations. Field locations included two conventionally tilled, monocultured cotton fields (ES and LM) and one no-till field following a year of corn production (LA). Greenhouse trials indicated that all commercial cotton varieties tested were susceptible to the reniform nematode. Sixty days after initial inoculation of 2000 vermiform nematodes, reproductive indexes ranged from 13 to 70 on Sure Grow 747 and Deltapine 424 BGII/RR, respectively. In the ES field, Stoneville 4793 RR produced 393 kg/ha more seed cotton per ha without a nematicide as compared to the nematicide treated plots. Seed cotton yields were not ($P < 0.05$) increased by the application of a nematicide for Fiber Max 991 RR and Fiber Max 991 BR. The yields for the remaining 29 varieties ($P < 0.05$) were increased in the nematicide treated plots as compared to the non treated plots. In the LM field, all varieties treated with the nematicide out yielded ($P < 0.05$) varieties without the nematicide. Following one year of corn production in the LA field, nematicides had no impact on yield for any variety.

EFFECT OF ROTATION CROPS ON *HETERODERA GLYCINES* POPULATION DENSITY IN A PRELIMINARY GREENHOUSE STUDY. **Vetter, S. A.,[1] S. Chen,[2] D. L. Wyse,[3] G. A. Johnson,[2] and P. M. Porter.[3]** [1]Department of Plant Pathology, University of Minnesota, Saint Paul, MN 55108; [2]Southern Research and Outreach Center, University of Minnesota, Waseca, MN 56093; [3]Department of Agronomy and Plant Genetics, Saint Paul, MN 55108.

Soybean production in Minnesota is greatly affected by the presence of the soybean cyst nematode, *Heterodera glycines*. A greenhouse study was carried out to determine the effect of crop sequence on population density of *H. glycines*. Seed of 38 plant species grown as field crops or cover crops in Minnesota were sown in 16-cm-diameter clay pots containing soil naturally infested with the soybean cyst nematode. The soil was obtained from field plots in Waseca, MN, and was mixed thoroughly for even inoculum distribution. The experiment was a randomized block design including the 38 crop treatments plus a no-crop control with six replicates. In the greenhouse, the soil was kept moist by watering every day. The pots were fertilized once during this time. Two and half months after planting, the crops were cut down to the soil level, the soil was mixed, a sample of the soil was removed, and the aboveground plant tissue was chopped and mixed into the soil for the next phase of the experiment. The soil samples were processed to determine nematode egg counts. Egg population density in the no-crop control did not differ significantly from that in most crop treatments. However, the *Brassica campestris* treatment resulted in higher egg population density than the control. As a group, legume crops resulted in lower egg population densities than monocots and *Brassica* species. Experiments are underway to determine the effect of plant residues on nematode population density and infectivity.

DEVELOPMENT OF MOLECULAR MARKERS FOR ROOT-KNOT NEMATODE RESISTANCE IN COTTON. **Wang, C., and P. A. Roberts.** Department of Nematology, University of California, Riverside, CA 92521.

Root-knot nematode (*Meloidogyne incognita*) is a major pest of cotton. Host-plant resistance is an economical, safe and effective method for managing nematodes in cotton. Three cotton cultivars (two of *Gossypium hirsutum* and one of *G. barbadense)* were characterized based on the phenotype of reactions to *M. incognita*. Two recessive genes in the nematode-resistant cv. NemX (*G. hirsutum*) were identified as the basis of resistance to *M. incognita* by genetic analysis of F_1, F_2, F_3, BC_1F_1 and F_7 recombinant inbred lines from the cross of NemX with susceptible *G. hirsutum* cv. SJ-2. Four AFLP markers tightly linked to the resistance trait were obtained by bulked segregant analysis (BSA) together with AFLP analysis of F_3 and F_7 populations. Genetic analysis of F_1, F_2 and BC_1F_1 progenies from the cross of NemX with susceptible G. barbadense cv. Pima S-7 showed a different inheritance of resistance from that in the NemX × SJ-2 cross, indicating that cv. Pima S-7 carries one of the NemX resistance genes. AFLP markers linked to the resistance are being screened in Pima S-7 × NemX progenies to confirm the resistance gene relationships and for genome mapping.

DESCRIPTION OF *BURSAPHELENCHUS CURVICAUDATUS* N.SP.(NEMATODA: APHELENCHOIDEDAE) ISOLATED FROM WOOD PACKAGING MATERIAL. **Wang, Jincheng, Benyuan Yu, and Maosong Lin.** Plant Pathology Department, Nanjing Agricultural University, Nanjing, 210095, China.

*Bursaphelenchus curvicaudatu*s n.sp. is described and illustrated. Specimens were extracted from the intercepted packaging wood from Lianyungang Port, Lianyungang city, China. *Bursaphelenchus curvicaudatus* n.sp. is characterized by relatively long body lengths (female 767-960 μm; male 663-831 μm), short stylets (female 13.9–17.4 μm; male 13.9–16.5 μm) with weak basal thickening, small spicules (16.5–21.6 μm) with obscure cucullus and distinguishingly bent female tails. Lots of features such as spicule shape, female tail shape, body length supported *Bursaphelenchus curvicaudatus* n.sp. distinguishes from *B. hofmanni, B. abietinus, B. fungivorus, B. hellenicus, B. hylobianum, B. rainulfi* and *B. eggersi* and *B. corneolus*. The PCR-ITS-RFLP pattern also provided further evidence that this isolate is a new species.

COMPARISON OF *CROTALARIA JUNCEA*ORGANIC FERTILIZER AND AMMONIUM NITRATE ON NEMATODE COMMUNITIES. **Wang, Koon-Hui,[1] R. McSorley,[1] A. Marshall,[2] and R. N. Gallaher.[2]** Department of [1]Entomology and Nematology, [2]Agronomy, University of Florida, Gainesville, FL 32611.

Impacts of organic versus inorganic fertilizers on nematode communities are variable, and depend on the specific organic fertilizer used. Field experiments were conducted during 2001 and 2002 in a squash (*1*Cucurbita pepo) agroecosystem to determine if applying sunn hemp (*Crotalaria juncea*) hay as an organic fertilizer improved nematode communities involved in soil nutrient cycling over an equivalent N rate (100 kg N/ha) of ammonium nitrate. Fertilizer source had no effect on nematode communities in 2001 when treatments were applied after winter cover crop of oat (*Avena sativa*), but differences (P £ 0.05) between the fertilizer sources occurred in 2002 when no winter cover cropping preceded squash. Soil fertilized with sunn hemp hay enhanced abundance of many bacterivore taxa, and increased fungivores at the end of the experiment. Compared to ammonium nitrate, fertilization with sunn hemp hay resulted in a community with lower maturity index, higher enrichment index, and lower channel index, indicating a disturbed and nutrient-enriched soil food web undergoing bacterial decomposition. Sunn hemp hay had little impact on omnivores and predatory nematodes, but suppressed plant-parasitic nematodes. Increasing sunn hemp hay rate to 200 kg N/ha increased abundance of bacterivores, fungivores, and predatory nematodes, and total nematode abundance compared to hay at 100 kg N/ha. Fertilization with ammonium nitrate increased percentage of herbivores, but reduced percentages and abundance of omnivores. In conclusion, sunn hemp fertilizer resulted in a greater stimulation of nematodes involved in nutrient cycling.

INUNDATIVE APPLICATIONS OF ENTOMOPATHOGENIC NEMATODES TEMPORARILY CHANGE POPULATIONS OF FREE-LIVING, SOIL NEMATODES**. Webster, J. M., and R. Chevalier.** Department of Biological Sciences, Simon Fraser University, Burnaby, Vancouver, BC, Canada, V5A 1S6.

When 200–250 infective juvenile (IJ) *Heterorhabditis* sp. were applied to the surface of a soil column, the IJs migrated down 15cm within 72h, as measured by a larval *Galleria mellonella* assay. Most free-living nematodes live in the top 15–20 cm, depending on soil type and species of plant roots. In the field, innundatively applied entomopathogenic nematodes are exposed directly or indirectly to many trophically or non-trophically related soil organisms. When *Heterorhabditis* IJs were applied at 0.38 million/sq.m to grass field plots or at 1.5 million/sq.m to plots in three different habitats, there were significant, temporal changes to the natural population densities of some free-living nematode genera, especially bacterivores. As well, populations of different nematode genera within the same nematode trophic group were affected differently. When heat-killed Heterorhabditis sp. IJs were applied (1.5 million/sq.m) to grassland plots, populations of some nematode genera were perturbed more than others, presumably due indirectly to soil enrichment from the decaying nematode cadavers. Variability in the data was probably due in part to the presence of nematode infected, insect cadavers and to isolated plant species causing aggregation of some nematodes, and making effective soil sampling difficult. Innundative releases of EPNs probably perturb the natural population fluctuations of edaphic, free-living nematodes only temporarily.

VIRULENCE OF *PARATRICHODORUS MINOR* AND *TRICHODOROUS PROXIMUS* TO WARM- SEASON TURFGRASSES IN FLORIDA. **Welch, J. K., and W. T. Crow.** Entomology and Nematology Department, University of Florida, Gainesville, FL 32611.

Trichodorus proximus and *Paratrichodorus minor* are the most common species of stubby-root nematodes found parasitizing turfgrasses in Florida. Better quantification of the amount of damage caused by these nematodes can aid in diagnostic and management efforts. In greenhouse experiments the reduction in root length caused by *T. proximus*, *P. minor*, and the unrelated ectoparasite *Belonolaimus longicaudatus*, were compared on bermudagrass (*Cynodon dactylon* hybrid) and St. Augustinegrass (*Stenotaphrum secundatum*). Separate experiments were performed for each grass species. The turf was grown in 1,500 cm3 clay pots and inoculated with either 400 *T. proximus*, 400 *P. minor*, 100 *B. longicaudatus*, or remained uninoculated. Each experiment used a completely randomized design with five replications and was repeated in two trials. After inoculation, the pots were placed in a climate-controlled greenhouse for 100 days. Roots were extracted from a 4-cm-diam. ×14-cm-deep core taken in the center of each pot and root lengths were evaluated using digital image analysis. The remaining soil was used for nematode reproduction analysis. Only *T. proximus* caused reductions in St. Augustinegrass roots in both trials ($P < 0.05$), whereas *P. minor* caused root reductions in only one trial. All three nematode species caused root reductions of bermudagrass ($P < 0.05$), but reductions were greater from *T. proximus* than *P. minor*. No differences ($P < 0.05$) in reproduction were detected between *T. proximus* and *P. minor* on either turf species. These results indicate that *T. proximus* may be the more damaging of the two common species of stubby-root nematode to turfgrasses in Florida.

TOWARD UNDERSTANDING ROOT-KNOT NEMATODE RESISTANCE IN TOMATO. **Williamson, Valerie and Chin-Feng Hwang.** Department of Nematology, University of California, Davis, CA 95616.

The tomato gene *Mi-1* which confers resistance against several root-knot nematode species, is a member of a large class of plant genes whose members are involved in specific resistance against pathogens including viruses, bacteria and fungi. Proteins encoded by these genes are thought to mediate pathogen recognition and signaling of host defense. *Mi-1.2*-mediated resistance is characterized by a hypersensitive response, consisting of localized cell death. Analysis of *Mi*-mediated defense has been extremely difficult because of the highly localized interaction site in the plant root. We have

produced a mutated version of *Mi-1, Mi-DS4*, that induces cell death about 3 days after the gene is transiently expressed in *Nicotiana benthamiana* leaves. There is strong correlation between amino acid substitutions that produce a loss of *Mi*-mediated nematode resistance in roots and a loss of cell death after transient expression of *Mi-DS4*. In addition, both *Mi-1*-mediated nematode resistance and *Mi-DS4*-mediated cell death are temperature sensitive, and the signaling molecule salicylic acid is required for both processes. We have used subtractive hybridization to identify genes that are induced upon transient expression of *Mi-DS4*. One of these encodes ACC oxidase, which catalyses the last step in ethylene biosynthesis. Cobalt chloride, an ACC oxidase inhibitor, blocks *Mi-DS4*-mediated cell death in *N. benthamiana* leaves and abolishes nematode resistance in tomato roots. This suggests that ACC oxidase is required for both *Mi-DS4*-mediated cell death and for *Mi-1.2*-mediated nematode resistance, and that analysis of genes induced upon *Mi-DS4* expression will provide insights into the signaling leading to nematode resistance.

IDENTIFICATION OF TROPHIC RELATIONSHIPS FOR FREE-LIVING MICROBIVOROUS NEMATODES VIA DNA EXTRACTION, AMPLIFICATION, AND SEQUENCING OF NEMATODE GUT CONTENTS. **Winkler, Kyle A., and B. J. Adams.** Department of Microbiology and Molecular Biology, Brigham Young University, Provo, UT 84604.

Species-specific food web relationships involving free-living microbivorous nematodes are not well established, yet critical to our understanding of their involvement in nutrient cycling and ecosystem function. Antarctic soil ecosystems are the simplest on earth and serve as a model system for exploring the role of biodiversity in ecosystem function. To establish which bacteria are involved in trophic relationships with Antarctic Dry Valley nematodes, we surface-sterilized nematodes and extracted their DNA, including bacterial DNA from the gut contents of the nematodes. From this bacterial DNA we PCR amplified the 16S gene and sequenced the product in order to determine the identity of the gut contents of the nematode. Given a broader sampling regimen, this approach can be used to establish food web relationships in more complex ecosystems.

PHYLOGENETIC RELATIONSHIPS AMONG *BURSAPHELENCHUS* SPECIES (NEMATODA: PARASITAPHELENCHIDAE) INFERRED FROM NUCLEAR RIBOSOMAL AND MITOCHONDRIAL DNA SEQUENCE DATA. **Ye, W. M.,[1] R. M. Giblin-Davis,[1] H. Braasch,[2] K. Morris,[3] and W. K. Thomas.[3]** [1]Fort Lauderdale Research and Education Center, University of Florida/IFAS, 3205 College Ave., Davie, FL 33314; [2] Federal Biological Research Centre for Agriculture and Forestry, Department for National and International Plant Health, Kleinmachnow Branch, Germany. Present address: Kantstrasse 5, D-14471 Potsdam, Germany; [3] Hubbard Center for Genome Studies, University of New Hampshire, 35 Colovos Rd., Durham, N H 03824.

A phylogenetic analysis using DNA sequences of the nuclear small and large subunit ribosomal RNA genes and mitochondrial cytochrome oxidase subunit I was performed. The analysis included representatives from 37 populations of 20 *Bursaphelenchus* species encompassing much of the known biological diversity in this genus. Phylogenetic analyses using several methods of inference were congruent, with the greatest resolution obtained with combined datasets. Phylogenetic analysis revealed *B. abruptus* as the basal taxon among all investigated *Bursaphelenchus* species and a large number of significantly supported monophyletic groups that are largely consistent with morphological and life history variation in the genus. Phylogenetic analysis of LSU rDNA sequences revealed the existence of cryptic species of *B. mucronatus*, *B xylophilus* and *B. sexdentati* and supports the novel nature of three previously unnamed species. While SSU, LSU and mtCOI gene sequences are useful for inferring phylogenetic relationships in this analysis, the LSU dataset was most informative across this genus. By contrast, the mtDNA data was limited by non-stationary base composition and apparent saturation above the species level.

RESULTS OF A LONGIDORID (NEMATODA: LONGIDORIDAE) SURVEY OF ARKANSAS, 1999–2001. **Ye, Weimin, and R. T. Robbins.** Department of Plant Pathology, University of Arkansas, Fayetteville, AR 72701.

During a 1999 to 2001 Arkansas survey a total of 828 soil samples from 242 sites in 37 counties were examined for the presence of Longidorid nematodes. Multiple samples were taken per site. Longidorids were recovered from 542 (65.5%) of these samples and included 142 sites with *Longidorus species* and 127 sites with *Xiphinema* species. From142 *Longidorus* sites the known species *L. breviannulatus* (8 sites), *L. crassus* (36), *L. diacturus* (36), and *L. fragilis* (7) were identified as well as newly described species *L. biformis* (14), *L. grandis* (5), *L. paralongicaudatus* (20), and *L. paravineacola* (5), and seven unidentified species (1 or 2 specimens each) were recovered. From127 *Xiphinema* sites three species *X. americanum sensu lato* (71), *X. bakeri* (33), *and X. chambersi* (23) were identified. No attempt was made to identify *X. americanum sensu lato* (*X. americanum* -group) species. Multiple longidorid species were found at many sites. Males of parthenogenetic species *L. diadecturus* (7 specimens, from 4 sites), *X. americanum-sensu lato* (4 specimens, from 3 sites), *X. bakeri* (10 specimens, from 2 sites), and *X. chambersi* (2 specimens, from 2 sites) were identified. Most samples were taken at a depth of 10 to 40 cm from sandy soil in the rhizosphere of hardwood trees. When extracting these very large nematodes care must be taken to avoid throwing them out with the debris on the coarsest sieve used.

EVASIVE BEHAVIOR OF WHITE GRUB SPECIES AGAINST ENTOMOPATHOGENIC NEMATODES. **Yoder, Corrie, and Parwinder S. Grewal.** Department of Entomology, The Ohio State University, OARDC, 1680 Madison Ave., Wooster, OH 44691.

Emphasis on biological alternatives to pesticides has increased in agriculture due to concern about environmental pollution. Entomopathogenic nematodes (EPNs) are used as biological control agents for soil dwelling insects with varying success. We hypothesized that grub species differ in their defensive and evasive behaviors, thus altering their ability to resist EPN attack. We evaluated the evasive behavior of *Rhizotrogus majalis* (EC), *Popillia japonica* (JB), *Cyclocephala borealis* (NMC), *Exomala orientalis* (OB), *Phyllophaga* sp. (JUB), *Maladera castanea* (AGB), and *Macrodactylus subspinosus* (RC) against *Steinernema scarabaei*, *Heterorhabditis bacteriophora* (GPS11), and *H. zealandica* (X1) under laboratory conditions in soil chambers. Grub movement after the addition of EPNs was marked directly on the chambers every 20 minutes for 2 hours. Grubs were then removed from the chambers and their subsequent mortality was recorded after 5 days. Mean distance traveled per 20-min increment, total distance in 2 hours, and percent larval mortality was quantified for each treatment. Behavioral responses varied by grub and nematode species and more resistant grub species did not always move the most. In some cases, a more generalized response was observed across treatments. Further tests will focus on aggressive behaviors that aid larvae in their defense against nematodes and will isolate stimuli detectable by grubs.

SUPPRESSION OF RENIFORM NEMATODE POPULATIONS WITH COTTON-CORN ROTATIONS. **Young, L. D., W. T. Pettigrew, H. A. Bruns, and S. R. Stetina.** USDA ARS Crop Genetics and Production Research Unit, Stoneville, MS 38776.

The reniform nematode, *Rotylenchulus reniformis*, has become the predominant phytoparasitic nematode on cotton (*Gossypium hirsutum*) in Mississippi and Louisiana. Corn (*Zea mays*) is a nonhost and has potential to reduce the nematode's population size. Corn as a rotation crop was evaluated in a field study conducted from 2000 through 2003 at Stoneville, MS. The experimental design used was a randomized block split-plot with eight replications. The main plots were crop rotations (continuous cotton, cotton-corn-corn-cotton, corn-cotton-corn-cotton, or continuous corn), and subplots were one of four cultivars of either corn or cotton. Subplots were 6 rows spaced 102 cm apart by 7.6 m long. Nematode populations in the center 2 rows of each subplot were determined at planting, mid season, and harvest. These same rows were harvested for yield determination. Nematode populations at planting in plots planted to cotton the previous season (mean 5364/L) exceeded the action threshold of 2200/L for Mississippi, regardless of rotation sequence. When cotton followed one season of corn, nematode populations rebounded by the end of the season. However, nematode populations remained below damaging levels throughout the season in cotton following two seasons of corn. The crop rotation effect was significant at $P = 0.06$ during 2003, when cotton lint yield from the cotton-corn-corn-cotton rotation was 194 kg/ha greater than yield from the continuous cotton plots. Rotation did not impact corn yield. At these nematode population levels, a rotation with at least two consecutive years of corn appears to be necessary to achieve reniform nematode suppression sufficient to increase cotton yield.

INFLUENCE OF SOIL CHARACTERISTICS ON THE ABILITY OF AN ALKALINE STABILIZED MUNICIPAL BIOSOLID TO SUPPRESS *MELOIDOGYNE INCOGNITA*. **Zasada, I. A.,[1] and M. Tenuta.[2]** [1]USDA, ARS Nematology Laboratory, Beltsville, MD 20705; [2]Department of Soil Science, University of Manitoba, Winnipeg, MB, Canada R3T 2N2.

We tested N-Viro Soil (NVS), an alkaline stabilized municipal biosolid, as a potential control agent for plant-parasitic nematodes. In preliminary studies, we observed differential survival of *Meloidogyne incognita* populations that appeared to be related to soil-specific effects of NVS on soil pH. NVS was toxic to *M. incognita* juveniles (J2) only in soils where the pH was raised to above 10.5, 24 hrs after amendment. Consequently, we identified soil properties that were conducive to pH increase by NVS. In greenhouse studies, NVS was applied to a set of soils with differing properties (texture, % organic matter, 1:5 soil:water pH, CaCO3 equivalent and titratable acidity). The soils had previously been inoculated with 10,000 J2 *M. incognita* per liter soil. The effect of NVS on soil pH varied with soil type; 3% w/w NVS increased soil pH to 10.1, 8.1, 7.9 and 9.2 in a loamy sand, sandy loam and two silt loam soils, respectively. Titratable acidity, a measure of the ease with which an alkalizing agent can increase pH of soil to 10.0, was the soil characteristic most closely associated with mortality of *M. incognita* J2 in the presence on NVS. Since high pH may be unfavorable for plant growth, we conclude that the application of NVS for *M. incognita* control is most appropriate in poorly buffered soils in which the pH will decrease in a short time frame after amendment.

HIRSUTELLA RHOSSILIENSIS: PATHOGENESIS RELATED PROTEASE AND QUANTITATIVE DETECTION IN SOIL. **Zhang, L. M., B. Wang, X. Z. Liu.** Key Laboratory of Systematic Mycology and Lichenology, Institute of Microbiology, Chinese Academy of Sciences, Zhongguangcun, Beijing 100080, P.R.China.

A novel subtilisin, designated as Hrp1 was purified from the nematophagous fungus *H. rhossiliensis* isolate OWVT-1.

Molecular weight of the protease Hrp1 was estimated to be around 32 kDa by SDS-PAGE. Its protease activity had a broad pH range and displayed over 90% between 6 and 11 with a peak at 9. The optimal temperature for activity was 75 °C. The enzyme was highly sensitive to PMSF. The amino sequence of N-terminal was AVIDTGVEASHPEF, which was similar to that of serine proteases of other nematophagous fungi. A specific and quantitative real-time PCR assay was developed for detecting and monitoring *H. rhossiliensis* in soils after its application. The TaqMan assay was able to detect as little as 100 fg of *H. rhossiliensis* DNA from pure culture in a 25-μl reaction volume, and the soil DNA extract with 40 conidia of *H. rhossiliensis* g^{-1} soil could be detected consistently. Real-time quantitative PCR detection was compared with parasitism assay to investigate the presence and abundance of *H. rhossiliensis* isolate OWVT-1 in autoclaved, microwave-heated, and natural soil 2 weeks after application of the fungus with different inoculation levels. The amount of *H. rhossiliensis* DNA quantified by real-time PCR and percentage of the second-stage juveniles (J2) of the soybean cyst nematode parasitized by the fungus was the highest in autoclaved soil, intermediate in microwave-heated soil, and the lowest in natural soil; and the fungal parasitism of J2 was positively correlated with the amount of DNA ($r^2 = 0.873$). The combination of real-time quantitative PCR and parasitism assay will provide effective way to study the ecology of the biocontrol agent in soil.