

ABSTRACTS

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EFFECTS OF OXAMYL, INSECT NEMATODES AND *SERRATIA MARSCENS* ON A POLYSPECIFIC NEMATODE COMMUNITY AND YIELD OF TOMATO. **M. M. M. ABD-ELGAWAD (1) and H. Z. M. Aboul Eid (2).** (1,2) Dept. Plant Pathology, Nematology Lab., National Research Center, El-Tahrir St., Dokki 12622, Giza, Egypt.

In a sandy loam soil, 24% liquid oxamyl sprayed on the shoots of tomato cv. Peto 86 at 10 and 31 days after planting decreased ($P = 0.05$) soil and root population densities of *Meloidogyne incognita* race 1 until harvest and attained the highest (163.7%) tomato yield relative to the untreated control. In other treatments, a liquid culture of *Serratia marscens* and a nematode suspension containing four Egyptian isolates of *Heterorhabditis bacteriophora* were applied separately through drip irrigation and increased the yield 121.2% and 160.2%, respectively, compared to the control. Yield increase in nematode-treated plots was due mainly to insect control. A month after nematode treatment, *M. incognita* population levels in soil were undetectable whereas those treated by oxamyl or the bacterium had their lowest soil population densities at season-end. The ring nematode appeared only early in the growing season and the stunt nematode was dominant in most treatments throughout the season.

THE USE OF POLYACRYLAMIDE GEL ELECTROPHORESIS OF SOLUBLE PROTEINS IN THE TAXONOMY OF EGYPTIAN HETERORHABDITID NEMATODES. **M. M. M. ABD-ELGAWAD (1) and M. A. Mohamed (2).** (1) Dept. Plant Pathology, Nematology Lab.; (2) Dept. Molecular Biology, National Research Center, El-Tahrir St., Dokki 12622, Giza, Egypt.

Five Egyptian isolates of heterorhabditid nematodes were compared by SDS PAGE with previously finger printing identified Egyptian *Heterorhabditis bacteriophora* and *H. indicus*. Two isolates were more closely related in the number of bands in the pattern than the rest though their common bands relate them to *H. bacteriophora*. The same observation applied to the other two isolates but their common bands relate them to *H. indicus*. The fifth isolate showed to some extent dissimilarity with both species. The relation among the isolates based on their geographic distribution and temperature profile activity was presented and discussed.

EVOLUTION OF PARASITISM AMONG NEMATODE-TRAPPING FUNGI. **D. AHRÉN and A. Tunlid.** Microbial Ecology, Ecology Building, Lund University, 223 62 Lund, Sweden. Dag.Ahren@mbioekol.lu.se.

The evolution of parasitism among a group of ascomycetes that use traps to infect nematodes is studied. These fungi are soil-living organisms and can grow both as saprophytes as well as parasites. To study the evolution of parasitism among the nematode-trapping fungi a molecular phylogenetic tree based on the 18S rDNA sequences was reconstructed. The tree is in concordance with the different trapping structures but not with traditional taxonomy. A large-scale sequencing project is underway to identify putative virulence factors in the nematode trapping fungus *Monacrosporium haptotylum*. By filtration of the fungus it is possible to investigate the differential gene expression of the infection structure compared to the mycelia of the nematode-trapping fungus. Results from a population study of a net forming nematode-trapping fungus, *Duddingtonia flagrans*, based on sequencing and AFLP techniques (Amplified Fragment Length Polymorphism) will be presented.

INFLUENCE OF ETHOPROP, FENAMIPHOS, CARBOFURAN, AND OXAMYL ON *HETERODERA AVENAE* POPULATIONS AND YIELD OF WHEAT. **S. M. AI-REHIAYANI (1).** (1) Department of Plant Protection, College of Agriculture and Veterinary Medicine, King Saud University, Al-Qassim Branch, Saudi Arabia.

Field trial was conducted to compare the effects of ethoprop, fenamiphos, carbofuran and liquid or granular oxamyl on yield of wheat (*Triticum aestivum* L.) in soil infested with *Heterodera avenae*. The granular nematicides were mixed with soil at seed sowing while the liquid oxamyl was applied as foliar application four weeks after wheat emergence. At harvest, numbers of white cysts recovered from roots were significantly reduced ($P=0.05$) in plots treated with Ethoprop, Fenamiphos, Carbofuran, and granular Oxamyl (8, 18, 39 and 33 cysts/10g roots respectively) when compared with untreated

plots (73). Wheat seed yield was increased ($P=0.05$) following nematicides treatments but was unaffected by the granular oxamyl application. Foliar-applied oxamyl did not reduce white cysts numbers on roots but it enhanced the greatest seed yield when compared to other treatments.

THE DEVELOPMENT AND INFLUENCE OF *ANGUINA TRITICI* ON WHEAT. **S. A. ANWAR (1), M. V. McKenry (1), A. Riaz (2), and M. S. A. Khan (2).** (1) Dept. Nematology, UC, Riverside, CA 92521; (2) Dept. of Plant Pathology, U. A. Agriculture, Rawalpindi, Pakistan.

The effect of initial inoculum density [P(i)] of *Anguina tritici* on growth of wheat and nematode reproduction was studied in the lath-house. Five holes were drilled in 10-cm-diam clay pots filled with sterilized sandy clay loam. Five inoculum rates comprised of P(i) = 1, 2, 3, 4 and 5 galls per plant were used. One seed of wheat cv. Pak-81 along with desired P(i) was planted into each hole. Pots were arranged in a completely randomized design with five replications. At harvest nematode reproduction, shoot and root dry weight and yield per plant were assessed. Nematode reproduction, plant growth and yield parameters were greatly influenced by the P(i) levels. Nematode reproduction was inversely proportional to P(i). Reproduction rates [P(f) / P(i) where P(f) = final number of J2 per new cockle] for P(i) 16,000, 32,000, 48,000, 64,000 and 80,000 J2 per plant were 1.77, 0.76, 0.44, 0.27 and 0.21, respectively. The size of new cockles was directly proportional to size of the cockle. Shoot and root dry weight and grain yield per plant were also inversely related to P(i).

REACTION OF WHEAT CULTIVARS TO *ANGUINA TRITICI*. **S. A. Anwar (1), M. V. MCKENRY (1), A. Riaz (2), and M. S. A. Khan (2).** (1) Dept. Nematology, UC, Riverside, CA 92521; (2) Dept. of Plant Pathology, U. A. Agriculture, Rawalpindi, Pakistan.

Wheat cultivars were evaluated for their interaction with *Anguina tritici*. Clay pots of 10-cm diameter containing sterilized sandy clay loam soil were planted with five seeds of each cultivar, and arranged in a completely randomized design with five replicates. Adjacent to each planted seed of wheat a cockle of wheat with ca 16,000 hibernating J2 were also added. Appearance of cockle disease symptoms was recorded. Eight cultivars including Chakwal-86, Barani-70, Rawal-87, WL-11, Blue Silver, Shalimar-88, Lu-26 and Punjab-87 exhibited twisting of leaves and P(f)/P(i) values in excess of 1. Five cultivars including Punjab-85, Rawal-87, Kohinoor-83, Barani-83 and Faisal-87 exhibited no foliar symptoms with a P(f)/P(i) lower than 1. Reproduction rates [P(f)/P(i)] were directly proportional to size of new cockles. Highest *A. tritici* reproduction was 1.74, which occurred on Chakwal-86 and was associated with cockles of 5-mm in diam. Punjab-85 produced cockles of 3.7-mm diam and P(f)/P(i) was 0.88. Number of cockles produced on each cultivar was increased as reproduction rates were increased.

AFLP ANALYSIS TO INVESTIGATE THE PARASITIC ABILITY OF THE SOYBEAN CYST NEMATODE, *HETERODERA GLYCINES*. **N. ATIBALENTJA (1), G. R. Noel (2), and L. L. Domier (2).** (1) Department of Crop Sciences, University of Illinois, and (2) USDA-ARS, Urbana, IL 61801, U.S.A.

Fifty *H. glycines* populations were obtained by subjecting populations of races 1 through 6, 9, and 14 to several cycles of selection on each of six sources of resistance to *H. glycines*, Peking, Cloud, PI88788, PI89772, PI209332, and PI90763. The selected nematode populations were then evaluated, using 15 different AFLP primer combinations, for potential markers associated with parasitic ability. A number of DNA fragments specific to some of the races have been identified. In particular, a 142-bp DNA fragment that is specific to race 5 of *H. glycines* has been isolated, cloned, and sequenced. Studies are underway to assess the potential of this fragment as a diagnostic probe to identify populations of race 5 from among other field populations of *H. glycines*.

SOYBEAN YIELD AND *HETERODERA GLYCINES* POPULATION DYNAMICS IN THE MIDWESTERN U.S. AND ONTARIO, CANADA. **N. ATIBALENTJA (1), G. R. Noel (2), P. A. Donald (3), H. Melakeberhan (4), T. R. Anderson (5), S. Chen (6), J. Faghihi (7), J. M. Ferris (7), C. R. Grau (8), D. E. Hershman (9), A. E. MacGuidwin (8), T. L. Niblack (3), R. D. Riggs (10), W. C. Stienstra (6), G. Tylka (11), and T. Welacky (5).** (1) Univ. Illinois; (2) USDA-ARS; (3) Univ. Missouri; (4) Michigan State Univ.; (5) AAFC-AAC; (6) Univ. Minnesota; (7) Purdue Univ.; (8) Univ. Wisconsin; (9) Univ. Kentucky; (10) Univ. Arkansas; (11) Iowa State Univ.

A 4-yr study evaluated the effects of conventional tillage vs. no-till, narrow, medium, and wide row spacing, resistance, and rotation on soybean yield and *H. glycines* reproductive factor (Rf). Yields were higher on 18-cm and 38-cm than on 76-cm wide rows in seven of nine states, but interactions occurred with year, rotation, and genotype. Yield of resistant soybean was greater than the susceptible in five states, and in two, yield was equal only in the second crop. Only in Ontario did row spacing affect Rf, with higher Rf on 18-cm than on 38-cm and 76-cm wide rows on susceptible soybean. In five states Rf was lower on the resistant than on the susceptible genotype. The effects of tillage methods on yield and Rf were inconsistent.

APPLICATION OF GEOSTATISTICAL TOOLS TO ASSESS THE SPATIAL DISTRIBUTION OF *HETERODERA GLYCINES*. **F. AVENDAÑO (1), F. J. Pierce (2), O. Schabenberger (3), and H. Melakeberhan (1)**. (1) Dept. of Entomology, Michigan State University, East Lansing, MI 48824; (2) Center for Precision Agricultural Systems, Washington State University, Prosser, WA 99350; (3) Dept. of Statistics, Virginia Tech, Blacksburg, VA 24061.

Geostatistical tools were used to quantify and map *H. glycines* spatial distribution at different scales of resolution to assess the potential for site-specific management of this pest. A nested sampling design was applied two years in two Michigan fields. Cysts were obtained from single-core soil samples collected at preplanting each year. Semivariograms of cyst density revealed spatial dependence varied from 30 m in one field to 3 m in the other. Kriging produced maps showed consistent patterns of cyst aggregation in large and small clusters both years, suggesting a possible role for site-specific management. Cyst density maps corresponded to soil type maps indicating soil texture may influence the spatial distribution pattern of this pest, suggesting that soil properties should be considered along with nematode occurrence and population density when making site-specific management decisions for *H. glycines*.

NEMATODE SECRETIONS: THE MAKINGS OF A PLANT PARASITE. **T. J. BAUM**. Dept. of Plant Pathology, Iowa State University, Ames, IA 50011.

Only a few members of the diverse nematode phylum have evolved to feed on living plants. What sets these parasites apart from non-parasitic nematodes? Besides obvious morphological features, there are molecular adaptations that mediate the intricate interactions between the parasite and the host plant. These molecular adaptations are referred to as parasitism genes. While the nematode's protrusible stylet and the large esophageal gland cells are the principal morphological adaptations, the proteins expressed from parasitism genes in the glands and secreted through the stylet are what makes a nematode a plant parasite. Several strategies have been employed to identify these stylet secretions and the parasitism genes encoding them. Following initial success with antibody methodologies, molecular technologies are now providing new insights into nematode parasitism genes. Among the identified parasitism genes are some that encode known enzymes as well as a growing number of genes that encode proteins with unknown functions. While only few of the putative parasitism genes have homologs in *Caenorhabditis elegans*, plant-parasitic nematodes also appear to have borrowed genes from microbes.

IN SITU CHARACTERIZATION OF AN ADIPOKINETIC HORMONE-LIKE GENE IN ROOT-KNOT NEMATODE. **S. BEKAL and K. N. Lambert**. Dept. Crop Sciences, University of Illinois, Urbana, IL 61801.

A cDNA clone isolated from *Meloidogyne javanica* cDNA library was found to share a homology with the insect adipokinetic hormone family of neuropeptides. The highest homology was found to a charged adipokinetic hormone of *Drosophila melanogaster* and *Phormia terraenovae*. These peptide hormones are known to control muscle contraction and fat metabolism in insects. *In situ* hybridization with a digoxigenin labeled riboprobe determined that the hormone gene is expressed in two cells in the anterior part of the metacarpus and also in two esophageal-intestinal cells. Currently, we are performing immunolocalization experiments to determine where the hormone is localized in the nematode. We are also performing microinjection experiments using the hormone itself, to determine if the nematode hormone has similar functions to that of the insect hormones.

CLONING AND CHARACTERIZATION OF NEW ESOPHAGEAL GLAND GENES IN ROOT-KNOT NEMATODE. **S. BEKAL and K. N. Lambert**. Dept. Crop Sciences, University of Illinois, Urbana, IL 61801.

Two *Meloidogyne javanica* cDNA clones were isolated and characterized from a gland cDNA library. When the two sequences were compared they showed a 75 percent similarity to each other. Southern blot data confirmed presence of the genes in *M. javanica* and *M. incognita* genomic DNA, indicating the genes are part of a small gene family. *In situ* hybridization results with a digoxigenin labeled riboprobe showed these genes are expressed in the nematode's esophageal glands, probably the subventral glands. Searches of protein and nucleotide databases have failed to find homologous sequences, indicating we have cloned two related but novel genes. Analysis of the deduced amino acid sequences predicts the presence of signal peptide, suggesting that both genes may encode secreted proteins. Currently, we are producing antibodies against these proteins to determine if they are secreted from the nematode upon infection of the plant.

EFFECT OF PEARL MILLET AND SORGHUM HYBRIDS ON *PRATYLENCHUS PENETRANS* POPULATIONS AND POTATO YIELDS IN QUEBEC. **G. BELAIR, Y. Fournier, and N. Dauphinais**. Agriculture and Agri-Food Canada, St-Jean-sur-Richelieu, Quebec, Canada J3B 3E6.

One-year and two-year-crop rotation experiments were conducted from 1998-2000 to assess the impact of grain sorghum (*Sorghum bicolor*), forage and grain pearl millet (*Pennisetum glaucum*) on *Pratylenchus penetrans* populations in four potato fields (*Solanum tuberosum* cv. Superior) in Quebec. These crops were compared to oats, the standard rotation crop. On one site, oats plots were doubled to include a fumigated control (band application of metham sodium at 124 L/ha). Forage millet and, to a lesser extent, grain millet significantly reduced the number of *P. penetrans* when compared to oats.

Grain sorghum increased nematodes populations similarly to oats. The suppressive effect of both millets was persistent in plots where potatoes were grown the following year. The best potato yields were recorded in plots previously grown in forage millet and were comparable or greater than those recorded in fumigated oats plots. Based on potato fields harvested in 2000, forage millet significantly increased by 10% the marketable potato yields when compared to nonfumigated oats plots.

SCREENING OF PEANUT GERMPLASM SEGREGATING FOR RESISTANCE TO THE ROOT-KNOT NEMATODE USING SCAR PRIMERS AND RFLP MARKERS. **I. F. BENDEZU (1), M. Burow (2), C. Simpson (3), and J. L. Starr (1).** (1) Department of Plant Pathology & Microbiology, Texas A & M University, College Station, TX 77483-2132; (2) TAES, Lubbock, TX 79401; (3) TAES, Stephenville, TX 76401.

Using a partial DNA sequence of the RFLP marker R2430E, two sequence characterized amplified region (SCAR) primers were designed and used as markers for resistance to the root-knot nematode *Meloidogyne arenaria*. DNA extracted from individuals of a BC2F2 segregating population, was used in a SCAR-PCR reaction with the primers amplifying two bands of approximately 1200 bp (resistant allele) and 280 bp (susceptible allele). It was possible to clearly distinguish the susceptible and the resistant phenotypes. The results were not different from data obtained with the RFLP-Southern analysis. This SCAR-PCR technology greatly reduces the time and effort required for screening peanut breeding lines for resistant phenotypes.

DETECTION AND ISOLATION OF A POTENTIAL ANALOG OF THE MI GENE FROM THE PEANUT ACCESSION GKP 10602 (ARACHIS DIOGOI HOEHNE) USING DEGENERATE PRIMERS. **I. F. BENDEZU and J. L. Starr.** Department of Plant Pathology & Microbiology, Texas A & M University, College Station, TX 77483-2132.

A nucleotide binding site (NBS) sequence was isolated from the peanut accession GKP 10602 (*Arachis diogenes* Hoehne) using a PCR approach with degenerate primers designed from the conserved NBS motifs found in the sequence of the Mi gene in tomato. The DNA fragment isolated from peanut shows some percentage of identity with the analog gene from tomato. The same pair of primers amplified similar DNA fragments of approximately 500 bp. from analogous sequence in other crops such as bean, pea (fam. Leguminosae) and corn (fam. Poaceae). This technology allows us to isolate analogue sequences for important genes in shorter times and with less expense. Additionally, successful engineering of transgenic varieties should be simplified by the conservation of key sequences, such as NBS, that are involved in regulation of gene expression.

ALL-TAXA BIODIVERSITY INVENTORY OF GREAT SMOKY MOUNTAINS NATIONAL PARK. **E. C. BERNARD.** Dept. Entomology and Plant Pathology, University of Tennessee, Knoxville, TN 37901-1071.

The All-Taxa Biodiversity Inventory (ATBI) of Great Smoky Mountains National Park (GSMNP), a 10 to 12-year project, was initiated in 1998, with the goal of identifying every living species in the Park. More than 200 scientists throughout the world are or will be participating. Nineteen biodiversity reference plots have been established in major plant associations within the Park for intensive inventory. Historical and unpublished records are being gathered to enhance the developing data base of species collected during the ATBI life span. An estimated 100,000 species live in GSMNP, but it is believed that less than 10% of the total has been recorded. The vast majority of unrecognized species are soil invertebrates, of which arthropods and nematodes likely constitute the greatest part. Nematode research in GSMNP currently is focused on entomopathogenic nematodes, commensal intestinal nematodes of millipedes, and previously collected soil nematodes now curated at The University of Tennessee, Knoxville. A web site has been established to disseminate authoritative knowledge of GSMNP nematodes.

ANALYSIS OF NEMATODE COMMUNITY STRUCTURE. **M. F. BERNEY and G. W. Bird.** Dept. of Ent., Mich. State Univ.

The use of colonizer-persister nematode community analysis models has been difficult in some temperate zone systems. Seasonal variation in the rate of new soil organic matter deposition has led to massive variations in bacterial populations. This has resulted in large numbers of opportunistic bacterial feeders, which may at times, represent up to 90% of the total nematode community. This is often not a function of successional stage of the plant community. Thus seasonal variability of new soil organic matter makes determining the basal characteristics of the food web very difficult. The removal of plant feeding nematode genera from the calculations presents an additional problem, as they at times may represent up to 50% of the nematode community. Plant feeding nematodes also reflect the plant community, and its relationship to the soil. We assert and demonstrate that in both unmanaged successional and agronomic systems, understanding the nematode community and its role as an indicator of the condition of the soil ecosystem, often requires the inclusion of the plant parasitic and plant associate nematode genera information. Additionally the timing of sampling efforts must take into account seasonal organic matter changes.

THE EFFECTS OF SOIL CHEMICAL AND PHYSICAL PROPERTIES ON SOYBEAN PATHOGENS IN ILLINOIS. **J. P. BOND, J. S. Russin, B. G. Young, and A. J. Hoskins.** Dept. Plant, Soil and General Agriculture, Southern Illinois University, Carbondale, IL 62901.

Heterodera glycines and the causal agent of soybean sudden death syndrome (SDS), *Fusarium solani* f. sp. *glycines*, are the most important soybean pathogens in Illinois. The interrelationship between soil properties, *H. glycines* and *F. solani* f. sp. *glycines*, was evaluated in a 3.5 ha field plot and in transects at multiple sites in southern Illinois. The field plot and two transects were established in an irrigated field. Two other transects were established in non-irrigated fields. Each of the four transects originated in an area of low disease expression and continued through areas of more severe disease. In the field plot and along transects, disease expression and properties of the soil were measured. Root infection by *F. solani* f. sp. *glycines* increased and population densities of *H. glycines* decreased as the bulk density of the soil increased. Initial and final population densities of *H. glycines* correlated positively with higher SDS severity. In the field plot, soybean yield correlated inversely with SDS severity and with initial and final population densities of *H. glycines*.

EXCITEMENT OF *S. CARPOCAPSAE* INFECTIVE JUVENILES BY EXPOSURE TO HOST CUTICLE. **S. BUSS, E. Perez, and E. Lewis.** VPI & SU Department of Entomology, Blacksburg, VA 24060.

Infective juvenile (IJ) entomopathogenic nematodes respond to hosts in different ways, according to their foraging strategies. *S. carpocapsae* IJs, which ambush hosts, respond to them in a hierarchical manner; exposure to host cuticle excites IJs to be attracted to volatile cues. Without exposure to host cuticle, nematode response toward volatile cues is weak. We determined the duration of the state of excitement elicited by exposure to cuticle. We exposed *S. carpocapsae* IJs to host cuticle for 30 min., washed them from the cuticle, and measured their subsequent response to volatile cues immediately after exposure and 30, 60, 120 and 240 min. later. After 60 min., IJs returned to their non-excited state. In the second experiment, we documented excitation by labeling amphids with the lectin, wheat germ agglutinin. IJ *S. glaseri* are attracted to volatile cues without pre-exposure to host cuticle. Their amphids were labeled by wheat germ agglutinin, whereas *S. carpocapsae* IJ amphids were not labeled. We hypothesized that *S. carpocapsae* amphids would be labeled by wheat germ agglutinin after the IJs had been exposed to host cuticle.

EVOLUTION OF HOST SEARCH STRATEGIES IN ENTOMOPATHOGENIC NEMATODES. **J. F. CAMPBELL.** USDA ARS Grain Marketing and Production Research Center, Manhattan, KS 66502.

Entomopathogenic nematodes in the families Heterorhabditidae and Steinernematidae are lethal insect parasites with an infective stage that actively seeks out new hosts. Within the genus *Steinernema*, there is considerable variation in the behaviors exhibited by host-searching infective juveniles (e.g., standing and jumping behavior, attraction to host associated volatile cues, area concentrated search in response to contact cues, vertical distribution in soil, etc.). This behavioral variation is correlated with variation along a continuum between ambush and cruise host-search strategies. Infective juvenile behavior was characterized and mapped onto a molecular phylogeny of the genus. This comparative approach enables hypotheses to be developed about the evolution of foraging strategy as well as the assembly of the suite of traits associated with different search strategies. It is proposed that the ancestral *Steinernema* infective juvenile was an intermediate forager and that both ambush foraging and cruise foraging specializations have evolved within this genus.

MOLECULAR, MORPHOLOGICAL AND THERMAL CHARACTERS OF *PRATYLENCHUS* SPECIES (NEMATODA: TYLENCHIDA) AND RELATIVES USING THE D3 SEGMENT OF THE NUCLEAR LS 28SRRNA GENE. **L. K. CARTA, A. M. Skantar, and Z. A. Handoo (1).** (1) Nematology Laboratory, USDA-ARS, Beltsville, MD 20705 USA.

Gene sequences of the D3 segment of the LS28SrRNA gene generated by our lab and two others were aligned with the Clustal W program and evaluated by the PAUP program using parsimony and maximum likelihood methods. Congruence of trees with thermal, vulval and lip characters was evaluated. When *Radopholus similis* was the outgroup, and ambiguously-alignable positions excluded, tree topologies were congruent with morphological taxa possessing 2, 3 or 4 lip annules. An updated sequence for *Pratylenchus hexincisus* indicated it was an outgroup of *P. penetrans*, *P. arlingtoni*, *P. fallax* and *P. convallariae*. *Pratylenchus zae* was a sister to *P. hexincisus*. The sisters of *P. teres* were *P. minyus* and *Hirschmanniella belli* rather than morphometrically similar *P. crenatus*. The *P. agilis* sequence is more closely related to the nearly identical sequences of *P. pseudocoffeae* and *P. brachyurus* than to *P. scribneri*, the originally diagnosed relative.

A NEW SPECIES OF *OSCHEIUS* (NEMATODA: RHABDITIDA) FROM FORMOSAN TERMITES (*COPTOTERMES FORMOSANUS*) AND A 28SRDNA MOLECULAR PHYLOGENY WITH RELATED TAXA. **L. K. CARTA (1), K. Morris (2), and W. K. Thomas (2).** (1) Nematology Laboratory, USDA-ARS, Beltsville, MD 20705 and (2) Department of Biology, University of Missouri, Kansas City 64110 USA.

A nematode isolated from Formosan termite heads in unthrifty colonies at the USDA Formosan Termite Research Unit, New Orleans, Louisiana, was determined to be a new species of the genus *Osccheius* Andrassy, 1974. The SEM face view showed three wide canals between paired lip sectors. The hermaphroditic population was compared to other live species

with morphometrics, mating and molecular characterization. Gene sequences of the D3 segment of the LS28SrRNA gene were generated for the new species and related species and genera such as *Dolichorhabditis* Andrassy, 1983. There were 15 apomorphic sites out of 277 alignment positions that characterized the new species compared to *O. insectivora*, *O. myriophila*, *O. necromena*, and two undescribed species.

PATTERNS OF NUCLEOTIDE SUBSTITUTION WITHIN THE MELOIDOGYNE RDNA D3 REGION. P. C. CHEN (1), P. A. Roberts (1), A. E. Metcalf (2), and B. C. Hyman (1). (1) University of California, Riverside, CA 92521; (2) Dept. of Biology, California State University San Bernardino, CA 92407.

Evolutionary relationships among a worldwide collection of *M. hapla* isolates and local isolates of *M. arenaria*, *M. incognita* and *M. javanica* were examined for nucleotide variation within the D3 26S rDNA region. Using D3A and D3B primers, a 350 bp region was PCR amplified from genomic DNA and double-stranded nucleotide sequence obtained. Phylogenetic analysis using 3 independent clustering methods all placed *M. hapla* in a separate clade from the other species, providing strong support for a division between automixis and apomixis. These affinities are based primarily on a 3-bp insertion at position 204 in *M. hapla* isolates. The 3 apomictic species share a common D3 haplotype indicating a recent branching. In *M. hapla*, single individuals contained 2 different haplotypes. Isolates of *M. javanica* appear to have fixed only one haplotype, while *M. incognita* and *M. arenaria* maintain more than one haplotype in an isolate. The D3 nucleotide polymorphism levels within *Meloidogyne* are further investigated by comparison to published studies.

EFFECTS OF BENZO-(1,2,3)-THIADIAZOLE-7-CARBOETHIOIC ACID S-METHYL ESTER ON REPRODUCTION OF ROTYLENCHULUS RENIFORMIS ON COWPEA AND PINEAPPLE. B. CHINNASRI, B. S. Sipes, and D. P. Schmitt. Dept. Plant and Environmental Protection Sciences, Univ. of Hawaii at Manoa.

The synthetic systemic acquired resistance inducer, benzo-(1,2,3)-thiadiazole-7-carboethioic acid S-methyl ester (BTH), was applied to cowpea and pineapple in the greenhouse to determine if reproduction of *Rotylenchulus reniformis* could be reduced. In cowpea, BTH applied to the leaves at 50 to 400 mg/L reduced the number of eggs produced by the nematode by 25 to 73% in 45 days. A single foliar application of BTH at 100 mg/L to 1-month-old pineapple plants 2 days after inoculation reduced nematode reproduction by 50% compared to that of an untreated control. BTH at these concentrations was not toxic to the plant nor the vermiform stages of *R. reniformis*. BTH may be stimulating the plants to express some resistance to the nematodes.

CHARACTERIZATION OF INBRED LINES OF HETERODERA GLYCINES. A. L. COLGROVE and T. L. Niblack. Dept. of Plant Microbiology and Pathology, Univ. of Missouri-Columbia.

Populations of *H. glycines* can be differentiated based on the ability of females to develop on *H. glycines*-resistant soybean, measured as a female index (FI). However, molecular identification of factors involved with FI is needed. A collection of *H. glycines* lines inbred by single-cyst descent and mass selection was developed to investigate differences in ability to develop on soybean cultivars with resistance. Some of these lines were derived from the same initial population but differ in ability to develop on specific soybean hosts, some have different lineages but similar patterns of development, and some have both different lineages and developmental patterns on resistant soybean. An AFLP protocol was developed for *H. glycines* and is being used to characterize the inbred lines and develop molecular markers for female development on specific soybean cultivars. Consistent polymorphisms found among and between the inbred lines can facilitate identification of markers linked to genes involved with female development on specific resistant soybean. Such markers can be used to characterize field populations currently identified as "races".

POTENTIAL MANAGEMENT OF COLORADO POTATO BEETLE WITH THE ENTOMOPATHOGENIC NEMATODE HETERORHABDITIS MARELATUS. N. COTTRELL, E. Grafius, and H. Melakeberhan. Dept. of Entomology, Michigan State University, East Lansing, MI 48824.

The objective of this study was to evaluate the pathogenicity of *Heterorhabditis marelatus* Liu and Berry to Colorado potato beetle (*Leptinotarsa decemlineata* Say), an important pest of potato that has considerable resistance to many insecticides. Field plots (322 square m), naturally infested with eight beetles per plant, and field cages (2 × 2 × 2 m), with nine plants each, infested with five beetles per plant, were treated with 0, 375, 750, or 1,200 infective juvenile nematodes/square m of soil. Nematode treatments were replicated four times. After nematode application, beetles were counted weekly for 7 weeks; numbers were significantly higher in control plots compared to nematode treated plots in both studies. There was also significantly more defoliation in control plots compared to nematode treated plots in both studies. Use of entomopathogenic nematodes could be integrated with other management tactics for control of Colorado potato beetle.

SURVEY OF PLANT-PARASITIC NEMATODES IN GOLF COURSES ALONG THE NORTHERN COAST OF THE GULF OF MEXICO. W. T. CROW (1) and S. D. Davis (2). (1) Entomology and Nematology Department, University of Florida; (2) Aventis Environmental Sciences.

Plant-parasitic nematodes are important turfgrass pests on golf courses in the southeastern United States. An unbiased survey of plant-parasitic nematodes from 42 golf courses located along the northern coast of the Gulf of Mexico including 17 courses in southeastern Louisiana (LA), southern Mississippi (MS), and northeastern Florida (FL), was conducted in December of 2000. Nematode samples were collected from 2 greens and 2 fairways from each course. *Mesocriconema* spp. were the most frequently observed plant-parasitic nematodes, being found in >95% of fairways and >80% of greens. *Belonolaimus longicaudatus* was found in 57% of FL fairways versus 29% of LA/MS fairways, and 36% of FL greens versus 18% of LA/MS greens. *Hoplolaimus* spp. were found in 39% of FL fairways versus 12% of LA/MS fairways, and 68% of FL greens versus 35% of LA/MS greens. *Meloidogyne* spp. were observed in 83% of FL fairways versus 84% of LA/MS fairways, and 65% of FL greens versus 41% of LA/MS greens. Other plant-parasitic genera observed were *Caloosia*, *Dolichodorus*, *Helicotylenchus*, *Hemicriconemoides*, *Hemicyclophora*, *Longidorus*, *Paratrichodorus*, *Pratylenchus*, *Trichodorus*, *Tylenchorynchus*, and *Xiphinema*.

MOLECULAR DIAGNOSTICS OF NEMATODES AS PART OF THE PEST-PATHOGEN COMPLEX IN CROPPING SYSTEM SOILS. J. CURRAN. CSIRO Entomology, PO Box 1700, Canberra, ACT 2601, Australia.

Conserved DNA sequence data is a reliable means of identifying plant-parasitic nematodes. rDNA, mtDNA and other "standard" gene regions have provided diagnostic markers for many of the economically important taxa. Rapid progress has been made with the cyst- and root-lesion nematodes, but the root-knot nematodes have proven less tractable. The latter perhaps reflects the more complex evolutionary relationships within root-knot nematodes and the challenges of using repetitive DNA sequences to unravel polyploid and often parthenogenetic (and clonal) lineages. Notwithstanding the challenges of establishing taxon boundaries, any diagnostic that is to have value in decision support needs to quantify the range of pests and pathogens found in the soil of cropping systems. Not surprisingly, no single gene region has proven to be useful in diagnosing all pests and diseases of interest. Thus a routine diagnostic for soil pests and diseases requires technological approaches that develop cost-effective test formats and are a compromise between taxon identification, quantification and the level of information required for decision support.

MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION OF A NEW *STEINERNEMA* SP. FROM CHINA. G. C. CUTLER (1), S. P. Stock (2), and J. M. Webster (1). (1) Dept. Biological Sciences, Simon Fraser University, 8888 University Dr., Burnaby, BC, Canada, V5A 1S6; (2) Dept. Nematology, University of California Davis, 1 Shields Ave., Davis, CA, 95616.

We report a new *Steinernema* species isolated from China and maintained through multiple passages in *Galleria mellonella* larvae. Morphological, ribosomal DNA (28S rDNA), and cross-breeding tests were used for diagnostic purposes. Additionally, 28S rDNA sequence data was used to assess phylogenetic relationships of this isolate with other *Steinernema* species. The new species is related to other morphologically similar species, *S. abbasi* and *S. carpocapsae*, differing in the morphology of the male (first-generation) genital structures and the position of the excretory pore, and by the tail length of the third-stage infective juvenile. Phylogenetic interpretation of the 28S rDNA sequences placed the new *Steinernema* species more closely with the clade that includes *S. abbasi*, *S. riobrave*, *S. ceratophorum* and *S. bicornutum*.

ABNORMAL MALES OF (*HETERODERA GLYCINES*) FROM ROOTS OF RESISTANT SOYBEAN CULTIVARS. M. DAROCHA, T. Anderson, and T. Welacky. Agriculture and Agri-Food Canada, GPCRC, Harrow, ON, N0R 1G0.

Studies of soybean cyst nematode (SCN) development on resistant soybean varieties have emphasized development of females and fecundity. Few studies have been made on the development of SCN males. The development of males may be important in the virulence and size of field populations. In a study to determine the effect of different levels of resistance on SCN populations and virulence, numbers of both males and females were monitored. Five soybean varieties differing in resistance were planted in pots containing field populations of SCN. After 10 d growth, seedlings were removed from pots and grown hydroponically for 10 additional days to facilitate collection of emerging males. Total number of males emerging from roots decreased with increasing resistance of varieties. Morphologically abnormal males that were shorter and thicker than normal males were also noted in the culture solutions. The abnormal males were sluggish and may not be effective in mating. The number of abnormal males increased with increasing variety resistance to SCN. Abnormal males may result from the inability to establish an effective feeding site.

GENE DISCOVERY IN THE SOYBEAN CYST NEMATODE. E. L. DAVIS. Department of Plant Pathology, North Carolina State University, Raleigh, NC 27695-7616.

Reverse-genetic and genomic strategies are being used to identify parasitism genes whose products are expressed in the esophageal gland cells and secreted through the stylet of the soybean cyst nematode (SCN) during plant parasitism. Genes encoding endogenous beta-1,4-endoglucanases (cellulases) were cloned from SCN and their sequence similarity suggests that some SCN parasitism genes may have been acquired via horizontal gene transfer. The cellulases are expressed and secreted by only the migratory stages of SCN. cDNA libraries have been constructed by RT-PCR using mRNA isolated

from the microaspirated contents of SCN esophageal gland cells at different stages of plant parasitism. Initial analyses have identified at least twenty different genes expressed in SCN gland cells during parasitism that encode putative secretory proteins. Some of these genes are expressed exclusively within the single dorsal or two subventral esophageal gland cells of SCN and are differentially regulated during plant parasitism. Expressed sequence tag (EST) and microarray analyses are being employed with the gland-cell cDNA libraries for comprehensive parasitism gene discovery in SCN.

INTERACTION OF LESION NEMATODE INFECTION AND WATER STRESS ON CORN. R. F. DAVIS (1) and H. J. Earl (2). (1) Dept. Plant Pathology; (2) Dept. Crop and Soil Sciences, University of Georgia.

Three water regimes and three rates of Telone II were evaluated in a split-plot design with six replications for their effect on corn yield, leaf area index, radiation use efficiency, and nematode population level. Water regimes were applied to whole plots to induce minimal, moderate, or severe drought stress. Rates of Telone II (0, 1, and 6 gal/A) were applied to subplots to create varying levels of lesion nematode (*Pratylenchus* sp.) infection. A severe drought persisted during most of the growing season. Analysis of variance indicated that leaf area index, radiation use efficiency, and yield differed significantly ($P \leq 0.05$) among water treatments with increased water resulting in increased leaf area, radiation use efficiency, and yields. Nematode population levels per gram of fresh root were not affected by water treatments or by Telone II fumigation, and there were no significant water stress by Telone II rate interactions. Telone II treatments did not significantly affect leaf area index, radiation use efficiency, or yield. An average of 44 lesion nematodes per gram of root at harvest appears to have been too low to cause measurable effects.

PHYLOGENETIC ANALYSES OF MELOIDOGYNE SSU RDNA. I. T. DE LEY (1), P. De Ley (1), A. Vierstraete (2), G. Karssen (3), M. Moens (4), and J. Vanfleteren (2). (1) Dept. Nematology, Univ. California, Riverside CA 92521 USA; (2) Vakgr. Biologie, Univ. Gent, Ledeganckstr. 35, 9000 Gent, Belgium; (3) Plant Protection Service, POB 9102, 6700 HC Wageningen, Netherlands; (4) Agric. Res. Center, 9820 Merelbeke, Belgium.

We inferred phylogenies from small subunit rDNA sequences of 12 species of Meloidogyne and 4 outgroup taxa (*Globodera pallida*, *Nacobbus abberans*, *Subanguina radiculicola* and *Zygotylenchus guevarae*). Alignments were generated manually from a secondary structure model, and automatically using Clustal X and with Treealign. Trees were constructed using distance, parsimony and likelihood algorithms in PAUP* 4.0b4a. Obtained tree topologies were stable across algorithms and alignments, supporting 3 clades: clade I = (*M. incognita* (*M. javanica*, *M. arenaria*)); clade II = *M. duytsi* and *M. maritima* in unresolved trichotomy with (*M. hapla*, *M. microtyla*); and clade III = (*M. exigua* (*M. graminicola*, *M. chitwoodi*)). Monophyly of ((clade I, clade II) clade III) was given maximal bootstrap support. *M. artiellia* was always sister taxon to this joint clade, while *M. ichinohei* was invariably placed with maximal support as basal taxon within the genus.

MAPPING SOYBEAN CYST NEMATODE FIELD DISTRIBUTION. P. A. DONALD (1), K. A. Sudduth (2), and N. R. Kitchen (2). (1) Dept. Plant Microbiology and Pathology; (2) USDA/ARS, University of Missouri, Columbia, MO 65211.

Soybean cyst nematode (*Heterodera glycines*) (SCN) is the most destructive pest to US soybean production. Current management techniques include crop rotation and use of resistant cultivars, but even these strategies can fail to achieve SCN levels below the damage threshold. Our goal was to detect relationships between soil variables and SCN population level. Such relationships might lead to focused procedures for detecting SCN trouble spots within fields. Two Missouri claypan soil fields were grid sampled and tested for complete soil fertility and texture variables and SCN egg population level. SCN egg population density was measured at planting and harvest during the soybean years of the corn-soybean rotation. The soil and SCN data were mapped to determine if SCN distribution was significantly correlated with any of the soil variables measured. The field entrance and perimeter had the highest SCN egg population levels in one study field. The lowest egg level was found in areas of shallow topsoil and correlated with low yield. SCN egg levels at planting were more closely associated with soil factors than egg levels at harvest.

INTEGRATED APPLICATION OF PAECILOMYCES LILACINUS, PASTEURIA PENETRANS AND CATTLE MANURE FOR CONTROL OF MELODOGYNE JAVANICA. B. N. DUBE. Department of Biological Sciences, University of Zimbabwe, P.O. Box MP 167, Mount Pleasant, Harare, Zimbabwe.

The integrated application of *P. lilacinus* (Pl), *P. penetrans* (Pp) and cattle manure in controlling *M. javanica* (Mj) on beans was evaluated at two sites, Harare Research Station (HRS) and at University of Zimbabwe (UZ). At both sites, the experimental layout was of a completely randomised block design. At HRS, 10-litre PVC microplots were arranged into 8 treatments of 12 replicates and at UZ 35-litre microplots were arranged into 8 treatments of 5 replicates. Pl was applied at rate of 5 g/litre of soil (5×10^8 cfu/g substrate), Pp at 100 mg/litre of soil and cattle manure at 1 tonne/ha. Nematode counts were made at planting (Pi), mid-season (Pm) and at harvest (Pf). Root galling and bean yield were assessed at (Pf). At both sites single applications of either Pl, Pp or cattle manure resulted in suppressed populations of Mj, reduced root

galling and increased bean yield. At HRS, bean yield from microplots treated with PI, Pp and cattle manure was increased by 40% compared to either 14%, 13% or 8% resulting from single applications of biocontrol agents.

SOIL TEXTURE AND THE EFFICACY OF *STEINERNEMA RIOBRAVE* AGAINST *DIAPREPES ABBREVIATUS*. L. W. DUNCAN, J. G. Genta, and J. Zellers. University of Florida, IFAS, CREC, 700 Experiment Station Road, Lake Alfred, FL 33850, U.S.A.

Experiments were conducted to determine the influence of soil texture on the efficacy of the nematode *Steinernema riobrave* against larvae of *Diaprepes abbreviatus*. In all experiments, weevil larvae buried in cages (30 cm depth) were exposed to infective juveniles (IJ) of *S. riobrave* by treating soil at field capacity with 20 IJ per square cm surface area. During 7 days in both field and microcosm (120 liter soil volume) experiments, *S. riobrave* killed between 70-80% of the insects buried in sandy soil, but only 4-17% of larvae buried in sandy clay loam soil. Mortality of untreated controls did not exceed 13%. Larvae permitted to move freely in 120 liter microcosms were killed at a rate similar to that of larvae held in cages. Efficacy of *S. riobrave* in microcosms of six autoclaved soils from citrus orchards in different regions of Florida was not correlated with the proportions of sand, silt or clay in the soils, but was directly related to the percentage of coarse sand in the soils.

CROP PRODUCTION SYSTEMS FOR NONCHEMICAL CONTROL OF *ROTYLENCHULUS RENIFORMIS*. R. E. Edgar (1), C. F. WEAVER (2), R. Rodriguez-Kabana (2), C. R. Taylor (3), and D. G. Robertson (2). (1) Edgar Farm, Deatsville, Alabama 36022; (2) Department of Entomology and Plant Pathology, Auburn University, AL 36849; (3) Department of Agricultural Economics, Auburn University, AL 36849.

A three-year field study was initiated in central Alabama to assess the efficacy of seven different cropping sequences for control of *Rotylenchulus reniformis* in cotton. Identical cropping schemes were used the first two years. This included both conventional and Bt cotton in monoculture, corn, grain sorghum and velvetbean used alone and corn and grain sorghum in combination with velvetbean. In the third year all plots were planted with Bt cotton except the one scheme utilizing traditional cotton. All rotation crops showed moderate reductions in nematode populations the first year and drastic reductions after two years. Nematode populations rebounded quickly following all cropping sequences when cotton was planted in the third year. Little difference was noted in traditional versus Bt cotton in either nematode populations or yield response. Highest cotton yields were obtained following two years of corn alone or corn plus velvetbean.

SITE-SPECIFIC MANAGEMENT OF NEMATODES: PITFALLS AND PRACTICALITIES. K. EVANS (1), R. M. Webster (2), P. D. Halford (1), A. D. Barker (1), and M. D. Russell (1). (1) Plant-pathogen Interactions Division, IACR-Rothamsted, Harpenden, Herts, AL5 2JQ, UK; (2) Agriculture and the Environment Division, IACR-Rothamsted, Harpenden, Herts, AL5 2JQ, UK.

The greatest constraint to potato production in the UK is damage by potato cyst nematodes (PCN) *Globodera pallida* and *G. rostochiensis*. Management of PCN depends heavily on nematicides, which are costly. Of all the inputs in UK agriculture, nematicides offer the largest potential cost savings with spatially variable application, and these savings would be accompanied by environmental benefits. We mapped PCN infestations in potato fields and monitored the changes in PCN population density and distribution that occurred when susceptible potato crops were grown. The inverse relationship between pre-planting population density and multiplication rate of PCN makes it difficult to devise safe spatial application procedures, especially when the pre-planting population density is just below the detection threshold. Also, the spatial dependence found suggests that the coarse sampling grids used commercially may produce misleading distribution maps.

EFFECT OF RESISTANT SOYBEAN LINES WITH PUSCN14 ON POPULATIONS OF SOYBEAN CYST NEMATODE. J. FAGHIHI (1), R. A. Vierling (2), V. R. Ferris (1), and J. M. Ferris (1). (1) Dept. of Entomology, Purdue University, West Lafayette, IN 47907-1158; (2) Indiana Crop Improvement Association and Dept. of Agronomy, Purdue University, West Lafayette, IN 47907-1150.

The high yield potential of SCN resistant PUSCN14 germ plasm (CystX) initially was reported in 1998 and reaffirmed in 1999 and 2000 in both SCN infested and non-infested fields. In an infested field (1999), an indeterminate CystX line yielded 4007 Kg/h while susceptible cultivars Williams 82 and Resnik yielded 2034 and 1896 Kg/h respectively. In 1998, the SCN soil population in this field was 28000 eggs/250cc soil, whereas it was 3600 eggs/250cc in fall 2000. In a long-term rotation study in field micro plots, a 99% reduction in SCN population was observed in the first year of rotation in plots with CystX lines in 1999, and a 91% reduction in another set of plots with CystX lines in 2000. When compared with other non-host crops under study, the largest reduction in SCN populations occurred under CystX lines. Greenhouse studies also showed that under CystX lines, populations of SCN decreased faster and further than they did under alfalfa, corn, wheat, clover, SCN resistant cultivars Jack and Hartwig, or fallow.

DOES THE *STEINERNEMA FELTIAE/XENORHABDUS BOVIENII* COMPLEX CONTROL *MELOIDOGYNE JAVANICA*? D. J. FALLON (1), H. K. Kaya (2), and B. S. Sipes (1). (1) PEPS, Univ. of Hawaii, Honolulu, HI 96822; (2) Dept. of Nematology, Univ. of California, Davis, CA 95616.

Steinernema feltiae and its symbiotic bacterium, *Xenorhabdus bovienii*, were tested as biological control agents for *Meloidogyne javanica*. A single application of 1×10^{10} CFUs of *X. bovienii* was applied to sterilized soil infested with 500 *M. javanica* J2s. *Xenorhabdus bovienii* reduced *M. javanica* penetration in cowpea by 48% in 100 cm³ soil, and egg production in tomato by 61% in 450 cm³ soil. Experimental replications showed inconsistent results. Exudates extracted from 4-day old *S. feltiae*-infected *Galleria mellonella* and applied to 8% w/w moist sand infested with 500 *M. javanica* J2s, significantly reduced root penetration in cowpea. The number of living *M. javanica* J2s in the sand was significantly greater in the *S. feltiae*-insect exudate treatment than in the noninfected insect exudate control after 4 days. This suggests that *M. javanica* root penetration was negatively affected. Our results demonstrate a reduction trend in *M. javanica* penetration by the *S.feltiae/X. bovienii* complex. However, the trends for *M. javanica* suppression are highly variable and therefore insufficient to suppress *M. javanica* at a field level.

EVALUATION OF SYSTEMIC AND NONSYSTEMATIC NEMATOCIDES FOR THE CONTROL OF THE ROOT-KNOT NEMATODE, MELOIDOGYNE JAVANICA. A. FARAHAT (1), S. Al-Rehiyani (1), and M. Belal (1).

(1) Plant Protection Department, College of Agriculture and Veterinary Medicine, King Saud University, Al-Qassim Branch, Saudi Arabia.

Four systemic nematicides namely, aldicarb, carbofuran (3,10% ai), oxamyl (granules, liquid), fenamiphos and the nonsystemic, ethoprop were evaluated for the control of the root-knot nematode, *M. javanica*. The experiment was conducted under the green house conditions on eggplant, *Solanum melongena* grown in 15 cm plastic pots filled with sandy soil. The concentrations used were 0.1 and 0.2 g / kg soil added as side dressing one week after nematode inoculation (except for ethoprop, which added a week before or after nematode inoculation). Results indicated that mocap accomplished the best significant reduction in the numbers of galls and egg-masses (86 – 96%, 65 – 91%; respectively). Aldicarb and fenamiphos came after ethoprop without significant differences. Oxamyl granules achieved moderate degree of nematode control. Results of carbofuran (3, 10% ai) were unsatisfactory. Oxamyl liquid, aldicarb and ethoprop, in that order, were the best in improving plant growth criteria.

NEMATODE FAUNAL PROFILES OF SOIL ECOSYSTEMS. H. FERRIS (1), T. Bongers (2), and R. G. M. de Goede (3).

(1) Department of Nematology, University of California, Davis CA 95616; (2) Laboratory of Nematology, Wageningen University, Wageningen, Netherlands; (3) Sub-department of Soil Quality, Wageningen University, Wageningen, Netherlands.

Nematodes are partitioned into functional guilds based on feeding habit, opportunistic response to environmental enrichment and sensitivity to perturbation. Abundance within each guild is weighted by the indicator importance of that guild for the enrichment or structure characteristic of the system. Soil foodwebs are categorized as enriched but unstructured (A), enriched and structured (B), resource-limited and structured (C), or resource-depleted with minimal structure (D). Systems differ in predatory regulation of opportunistic taxa, amount of sequestered carbon, level and nature of enrichment, decomposition channels, and C:N of organic input. Category A systems are represented by annual-cropped agriculture with frequent disturbance; B systems by organically-driven, relatively undisturbed perennial systems; C systems by grasslands and forests with little disturbance or extrinsic enrichment; and D systems by stressed annual agricultural systems and contaminated sites.

MOLECULAR BARCODES FOR SOIL NEMATODE IDENTIFICATION. R. M. FLOYD, A. Papert, and M. L. Blaxter. ICAPB, University of Edinburgh.

We have developed a molecular barcoding system for identification of soil nematodes by DNA sequencing. PCR is carried out on individual nematodes, and the 5' segment of the small subunit ribosomal RNA (SSU) gene is amplified and sequenced. Resulting sequences (typically 450-500 bases) are aligned and clustered using a neighbour-joining algorithm. Groups of similar or identical sequences are designated as molecular operational taxonomic units (MOTU). A Scottish upland *Agrostis-Festuca* grassland soil was sampled, using both random and culture-based methods. The MOTU discovered could readily be assigned to classical, morphologically defined taxonomic groups using a database of SSU sequences from named nematode species. The MOTU technique allows a rapid assessment of nematode taxonomic diversity in soils. Correlation with a database of sequences from known species offers a route to application of the technique in ecological surveys addressing biological as well as genetic diversity.

CLONING PUTATIVE PARASITISM GENES EXPRESSED IN THE ESOPHAGEAL GLAND CELLS OF THE SOY-BEAN CYST NEMATODE. B. GAO (1), R. Allen (1), T. Maier (2), E. L. Davis (3), T. J. Baum (2), and R. S. Hussey (1).

Depts. of Plant Pathology, (1) University of Georgia, Athens, GA 30602; (2) Iowa State University, Ames, IA 50011; (3) North Carolina State University, Raleigh, NC 27695-7616.

Secretions produced in nematode esophageal gland cells play key roles in plant parasitism. Contents microaspirated from the gland cells and from the intestinal region of parasitic stages of the soybean cyst nematode were used to construct

separate cDNA libraries. Suppression subtractive hybridization (SSH) between gland cell and intestinal cDNA isolated genes expressed preferentially in the glands. SSH product was used to construct a gland cell library macroarray containing 3,000 cDNA clones. Gland contents were also used to construct a full-length cDNA library macroarray, representing 6,144 clones. Twenty-three unique cDNA sequences were identified from the SSH macroarray and full-length cDNAs of 21 of these clones were obtained from the full-length cDNA macroarray. Proteins encoded by 10 clones contained a signal peptide and PSORT II predicted 8 to be extracellular. mRNA in situ hybridization localized several clones within the dorsal or subventral glands of the nematode.

BIOLOGICAL SOIL SUPPRESSION AFFECTS BOTH SEXES OF *HETERODERA SCHACHTII*. X. GAO and J. O. Becker. Department of Nematology, University of California, Riverside, CA 92521.

In a local soil suppressive for beet cyst nematode, previous studies have suggested that fungal parasitism of females, cysts and eggs caused the nematode population decline. This project focussed on the population of males, as mating is required for successful reproduction of *H. schachtii*. Pots with five week-old seedlings of the host plant Swiss chard were infested with 2000 second-stage juveniles and incubated at 20°C in an environmental growth chamber. After the nematodes had completed two generations, the soil was amended with 20 percent (v/v) suppressive or conducive soil. *H. schachtii* populations were assayed at 200 and 400 degree days after amendment application. Both male and cyst populations were significantly less abundant in suppressive than in conducive soil at both sampling dates. The results indicate that a reduction in the male population may contribute to the *H. schachtii* population decline in the suppressive soil.

EVOLUTION OF PARASITISM IN INSECT-TRANSMITTED PLANT NEMATODES. R. M. GIBLIN-DAVIS (1), K. A. Davies (2), K. Morris (3), and W. K. Thomas (3). (1) Univ. of Florida, Davie, FL 33314, (2) Adelaide Univ., S.A., 5064, and (3) Univ. of Missouri, Kansas City, MO 64110.

In the Aphelenchida (Aphelenchoididae), *Bursaphelenchus cocophilus*, the *B. xylophilus* group, and *Schistonchus* spp. probably evolved independently from dauer-forming, mycophagous ancestors that were phoretically transmitted to breeding sites of their insect hosts in plants. Mycophagy probably gave rise to facultative or obligate plant parasitism because of opportunities due to insect host switches or peculiarities in host behavior. In the Tylenchida, there is one significant radiation of insect-associated plant parasites involving *Fergusobia* nematodes (Neotylenchidae) and *Fergusonina* flies as mutualists that gall myrtaceous plant buds or leaves. These dicyclic nematodes have different phases that are parasitic in either the insect or the plant hosts. Sequence comparisons within flies (mtDNA) and nematodes (rRNA) show a high degree of host specificity. The evolution of this association is unclear, but phylogenetic hypotheses are being generated from rDNA sequence data from *Fergusobia* and contemporary insect-parasitic and plant-parasitic forms in the Hexatylinea.

HOT WATER AND OZONE TREATMENTS OF EASTER LILY FOR MANAGEMENT OF LESION NEMATODE, *PRATYLENCHUS PENETRANS*. D. D. GIRAUD (1), B. B. Westerdahl (2), L. J. Riddle (3), C. E. Anderson (2), and A. Pryor (4). (1) University of California (UC), Cooperative Extension, Eureka, CA 95503; (2) Dept. Nematology, UC, Davis, CA 95616; (3) Easter Lily Research Foundation, Brookings, OR 97415; (4) SoilZone, Inc., Davis, CA 95616.

Easter lily bulbs for greenhouse forcing are produced in Del Norte County, CA and Curry County, OR. *P. penetrans* infestation of soil and roots is a serious detriment to production. In 3 years of field trials, hot water(HW) and ozone(O3) treatments of bulblet planting stock were tested alone, and in combination with commercial chemical standards and compared to untreated controls. Each trial consisted of 3 replicates of 40 treatments. Several treatments performed better than untreated, but not as well as commercial standards in all evaluation criteria. For example, HW treatment at 49° centigrade(C) for 35 minutes(M) or 46 C for 90 M, consistently reduced nematode populations within roots but did not substantially improve growth of bulbs. In contrast, O3 that was produced by a conventional electrical discharge generator did not reduce nematode numbers but improved bulb growth in some treatments.

ENDOGLUCANASE EXPRESSION IN PLANT-NEMATODE INTERACTIONS. M. GOELLNER, X. Wang, and E. L. Davis. Dept. of Plant Pathology, North Carolina State University.

The formation of specialized feeding cells within host roots by cyst and root-knot nematodes, termed syncytia and giant-cells, respectively, requires extensive cell wall remodeling. Cell wall degrading enzymes have been implicated in cell wall modifications observed during feeding cell formation; however, it is unclear whether the enzymes are of nematode or plant origin. Antisera specific to beta-1,4-endoglucanases (EGases) produced by the tobacco cyst nematode localized secreted nematode EGases in plant cortical tissue during migration, but not within syncytia. Tobacco EGase expression was upregulated in both root knot and cyst nematode-infected roots. Three full-length tobacco cDNA clones each encoding structurally divergent EGases belonging to glycosyl hydrolase family 9 have been characterized. *In situ* mRNA hybridization localized tobacco EGase transcripts within syncytia, giant-cells, root tips, and lateral root primordia. Tobacco EGases are likely an integral component of a complex array of cell wall enzymes recruited during nematode parasitism for the development of feeding cells in plant roots.

R-GENE HOMOLOGS IN POTATO CONFER RESISTANCE AGAINST DISTINCT PATHOGENS: NEMATODES AND VIRUSES. **A. GOVERSE (1), E. Bakker (1), G. Smant (1), H. Sandbrink (2), E. van der Vossen (2), and J. Bakker (1).** (1) Lab. Nematology, Wageningen Univ., the Netherlands; (2) BU Genomics, Plant Research International, the Netherlands.

Genome analysis of a single R-gene cluster in potato led to the isolation of two highly homologous resistance genes: *Gpa2* and *Rx1*. Both genes confer resistance to completely unrelated pathogens, namely the potato cyst nematode *Globodera pallida* and potato virus X. To get more insight into the evolutionary relationship between the two genes, new homologues were identified. All the homologues mapped to the same cluster on chromosome 12 of the diploid potato clone SH83. Sequence data of the homologues were used to perform phylogenetic analysis of this R-gene cluster. Sequence conservation between the effector domain of *Gpa2* and *Rx1* indicate the activation of a similar resistance mechanism. However, infection studies and histological observations showed a relatively slow resistance response for *Gpa2*, while *Rx1* results in extreme resistance. To gain more insight into the mechanism underlying nematode and virus resistance, structure-function analysis of *Gpa2* and *Rx1* are currently under investigation.

EVOLUTION OF MOLLUSK PARASITISM IN NEMATODES. **P. S. GREWAL, S. K. Grewal, and L. Tan.** Department of Entomology, Ohio State University, Wooster, OH 44691.

Although, there are no confirmed fossil records of mollusk parasitic nematodes, diverse associations of a wide array of nematodes with slugs and snails provide a fertile ground for speculation of the evolution of mollusk parasitism in nematodes. Slugs and snails are often recorded as intermediate hosts to nematode parasites of vertebrates. However, there are many nematode species that appear to be obligate parasites of slugs and snails. Studies of nematodes associated with slugs and snails reveal that bacteria-feeding nematodes in the genera *Caenorhabditis*, *Panagrolaimus*, *Diplogaster*, and *Phasmarhabditis* colonize the shell cavity under the mantle. The discovery of *Rhabditiophanes* in slugs, a nematode believed to be an ancestral form of entomopathogenic nematodes in the family Steinernematidae suggests that similarities between insect and mollusk parasitism in nematodes. Based on morphological and life history studies of the bacteria-feeding nematodes associated with slugs, hypotheses are formed on how the obligate parasitism of mollusks might have evolved in nematodes.

IMPACT OF GREEN MANURE CROPS ON SUSTAINABLE MANAGEMENT OF SUGAR BEET CYST NEMATODE. **S. L. HAFEZ and P. Sundararaj.** University of Idaho, Parma Research and Extension Center, 29603 U of I Ln, Parma, Idaho 83660, USA.

A series of experiments were conducted for three years to study the efficacy of green manure crops for management of sugarbeet cyst nematode, *Heterodera schachtii*, under field conditions. In all experiments, oil radish and white mustard cultivars were seeded at the rate of 25 lb/acre in fall and incorporated twelve weeks later as the field was prepared for sugarbeet. In the first year, mustard 'Concerta' produced 35% more above ground biomass than radish 'Colonel' and the viable cysts declined 29% and 19% in oil radish and mustard treatments, respectively. Planting of oil radish and mustard produced significantly more beet yield and sugar per acre than the untreated check. In the second year, radish 'Adagio' produced significantly more above ground biomass than mustard 'Metex'. Sugar beet yield from the green manure planted plot was significantly higher than the fallow plot. Planting of Adagio as a previous crop gave more beet yield than 'Metex'. In the third year, biomass (top, root and total) production of oilradish 'Dacapo' was significantly higher than mustard 'Metex'. A significant increase in sugar beet yield has been recorded following of oilradish and mustard, compared to fallow.

EFFICACY OF OIL SEED MEALS FOR MANAGEMENT OF *HETERODERA SCHACHTII* AND *MELOIDOGYNE CHITWOODI* UNDER GREEN HOUSE CONDITIONS. **S. L. Hafez and P. SUNDARARAJ.** University of Idaho, Parma Research and Extension Center, 29603 U of I Ln, Parma, Idaho 83660, USA.

Two experiments were conducted under green house conditions to find the effect of rapeseed, soybean and cotton seed meals on sugar beet cyst nematode *Heterodera schachtii* and Columbia root knot nematode *Meloidogyne chitwoodi*. For the experiment with *H. schachtii* 6" diameter plastic pots were filled with field soil naturally infested with *H. schachtii* (17 viable cysts with an average 95 eggs and larvae per cyst) and thoroughly mixed with each seed meal (10.4 g/pot equivalent to 2t/A of field). Sugar beet was planted in each pot at 10 seeds/pot and thinned to 3 plants/pot. The experiment with *M. chitwoodi* was conducted with the same treatments but the soil was inoculated with *M. chitwoodi* (4,500 J2) and four week-old tomato seedlings were planted in each pot (one/pot). In the first experiment three oil meals significantly reduced the *H. schachtii* larva, viable cysts on the root, and total nematode population. Highest root weight was observed in plants treated with rape meal. In the second experiment, all meals significantly reduced the *M. chitwoodi* larval population in the soil and root. Highest fresh and dry root and shoot weight were observed in rapeseed meal treatment.

A STRATEGY FOR CONTROLLING PLANT PARASITIC NEMATODES WITH A MODIFIED CRY6A. **K. HALE, J. Z. Wei, and R. V. Arioan.** Section of Cell and Developmental Biology, Division of Biology, University of California, San Diego, La Jolla, CA 92093-0349.

We are attempting to control plant parasitic nematodes with *Bacillus thuringiensis* (*Bt*) toxins. *Bt* toxins will be expressed in *Arabidopsis* or tomato roots and challenged with *Meloidogyne incognita*. We found Cry6A *Bt* toxin is toxic to phylogenetically diverse free-living nematodes. To minimize feeding exclusion effects of plant parasitic nematodes, we delineated the minimal Cry6A protein that is toxic. *Arabidopsis* plants have been transformed with a modified Cry6A gene under control of three different promoters. Currently, successfully transformed plant lines are being isolated and our nematode infection assays are being perfected. We are growing *Arabidopsis* plants in modified Knop medium and are infecting them with active J2's under sterile conditions. We will compare *M. incognita* infection rates of plants transformed with Cry6A versus nontransformed plants. Alternatively, we are investigating using the hairy root system to express the protein and test for effective control of plant parasitic nematodes.

TOLERANCE OF SUGAR BEET TO *HETERODERA SCHACHTII*. **J. HALLMANN (1), J. Schlang (2), K. Gierth (1), J. Muller (2), and R. A. Sikora (1).** (1) Institut für Pflanzenkrankheiten, Nussallee 9, D-53115 Bonn, Germany; (2) BBA-Institut für Nematologie und Wirbeltierkunde, Toppeideweg 88, D-48161 Münster.

Plant strategies directed to avoid damage caused by plant parasitic nematodes include resistance and tolerance. Especially plant tolerance becomes an important feature when later storage organs become infected by nematodes at an early stage, e.g. tap roots of sugar beet. In field studies and greenhouse experiments sugar beet cultivars were studied for their tolerance to *Heterodera schachtii*. With increasing nematode pressure yield decrease in tolerant sugar beet cultivars was significantly less than for intolerant cultivars. Tolerant plants suffered later from water stress and the photosynthetic rate was generally higher. Under greenhouse conditions, shoot weight was a reliable indicator for tolerance, and tolerant sugar beets showed a better compensatory root growth. The concept of tolerance for managing plant parasitic nematodes is discussed.

TERRAPY, A PLANT HEALTH PROMOTING AGENT WITH NEMATODE CONTROL POTENTIAL. **J. HALLMANN (1), J. Mulawarman (1), D. Bell (2), B. Kopp-Holtwiesche (2), and R. A. Sikora (1).** (1) Institut für Pflanzenkrankheiten, Nussallee 9, D-53115 Bonn; (2) Cognis Deutschland GmbH, Henkelstrasse 67, D-40551 Dusseldorf.

TerraPy is a plant health promoting agent. TerraPy has stimulating impact on soil microbial activity thereby accelerating nutrient availability and enhancing plant growth and health. The effect of TerraPy is especially pronounced on degraded and imbalanced soils. Tomato seedlings (*Lycopersicon esculentum* cv. Hellfrucht Frühstamm) were treated with 200 kg/ha TerraPy and subsequently inoculated with 3,000 eggs of *Meloidogyne incognita*. Population densities of soil bacteria and fungi, as well as saprophytic and plant-parasitic nematodes were determined 0, 1, 3, 7 and 14 days after treatment. After 14 days tomato shoot and root fresh weight as well as nematode infestation was recorded. TerraPy significantly enhanced soil microbial activity and affected species pattern. The number of saprophytic nematodes in the soil increased, whereas root-knot infestation was reduced. Plant growth increased >50% over the controls. TerraPy is concluded to be an interesting tool for use in managing nematode populations and root health.

DEVELOPMENTAL TEMPERATURE AND LENGTH OF *STEINERNEMA FELTIAE* JUVENILES (NEMATODA: STEINERNEMATIDAE). **S. Hazir (1), S. P. STOCK (2), H. K. Kaya (2), A. M. Koppenhofer (3), and N. Keskin (1).** (1) Dept. Biology, Faculty of Science, University of Hacettepe, 06532 Beytepe, Ankara, Turkey; (2) Dept. Nematology, University of California Davis, One Shields Ave. Davis, CA 95616; (3) Dept. Entomology, Rutgers University, New Brunswick, NJ 08901.

The development of five geographic isolates of *Steinernema feltiae* at 8, 15 and 23 C in wax moth, *Galleria mellonella*, larvae was examined. The isolates were from Mediterranean (Sinop from Turkey, SN from France, and Monterey from California), subtropical (Rafaela from Argentina), and tropical (MG-14 from Hawaii) regions. All isolates produced infective juveniles with longer body lengths at 8 C followed by 15 and 23 C. To further verify body length at the different temperatures, beet armyworm, *Spodoptera exigua*, larvae and dog-food agar medium were used, respectively, for in vivo and in vitro culture of the Sinop isolate. Infective juvenile body length showed the same trends with the longest being at 8 C and decreasing in length from 15 to 23 C. The data suggest that quality of food for the nematode and temperature (that is, developmental time) influence the body length of the infective juveniles.

ROLE OF ENDOPHYTE-INFECTED FESCUE FOR NEMATODE SUPPRESSION IN WEST VIRGINIA ORCHARDS. **J. R. HENDRICKS and J. B. Kotcon.** Div. of Plant and Soil Sci., West Virginia Univ., Morgantown, WV 26506.

Tall fescue (*Festuca arundinacea*) infected with the endophytic fungus *Neotyphodium coenophialum* was compared with endophyte-free tall fescue and bare fallow ground cover to assess the effect on three plant parasitic nematodes, predacious nematodes and naturally occurring nematophagous fungi. Endophyte-infected (cv. Kentucky-31) and endophyte-free (cv.

Stargrazer) tall fescue averaged 52% and 21% infection rates, respectively. Significantly lower population densities ($P < 0.05$) of *Pratylenchus crenatus* were observed in Kentucky-31 at 60 and 240 days after planting (DAP), a 62 and 70 percent reduction from that in Stargrazer. The same trend was observed in samples 120, 180 and 360 DAP but differences on these dates were not statistically significant. Populations of *Xiphinema americanum*, *Paratylenchus projectus*, predacious nematodes and nematophagous fungi did not vary significantly among treatments.

STREPTOMYCES SPP. COLONIZE MELOIDOGYNE ARENARIA EGGS "IN VITRO". T. E. HEWLETT, L. P. Norris, M. L. Smither-Kopperl, and J. H. White. Entomos LLC, 4445 SW 35th Terr., Suite 310, Gainesville, FL 32608.

Streptomyces spp. are common soil bacteria that produce both chitinases and antibiotics. It is believed that chitinases in soil can suppress populations of plant pathogenic nematodes because nematode egg shells contain chitin. This study examined the effect on egg hatch of *Meloidogyne arenaria* race one by *Streptomyces* isolates 7, 47, 54, 98 and 198. Spores of each isolate were plated on water agar, and approximately 50 nematode eggs were pipetted onto each plate. Plates were observed daily and after 7 days juveniles were washed from plates to determine percent hatch. Each treatment was replicated five times. In the first trial, isolates 47, 54, and 198 significantly decreased egg hatch 50, 55, and 55% respectively. In a repeat of this experiment isolates 7 and 98 were included. Isolates 47, 54, 98 and 198 significantly reduced egg hatch 24, 41, 52 and 26% respectively. Isolates 47 and 54 were observed to parasitize eggs that were in the early stages of development. Eggs with juveniles fully developed inside were not parasitized.

MAGNESIUM PARTITIONING IN COFFEA ARABICA INFECTED WITH MELOIDOGYNE KONAENSIS. D. R. HURCHANIK (1), D. P. Schmitt (1), and N. V. Hue (2). (1) PEPS, University of Hawaii; (2) TPSS, University of Hawaii.

The Kona coffee root-knot nematode (*Meloidogyne konaensis*) and soil fertility are limiting factors for optimal coffee (*Coffea arabica* L. cv. Typica selection Guatamala) growth in Kona on the island of Hawaii. Magnesium (Mg) partitioning was determined in the greenhouse for coffee grown in 10-cm-diam pots containing a Kealakekua Andisol soil. Soil was infested with 0, 75, 300 and 1500 J2 (Pi densities) of *M. konaensis*. Coffee infected with 0, 75, and 300 J2 had Mg levels in the leaves considered adequate (0.30 - 0.50 percent (%)) for optimum plant growth. At Pi 1500, Mg concentrations were marginal for optimum plant growth. There were negative linear relationships between Mg concentrations in leaves and roots with increasing Pi densities. Regression coefficients indicated *M. konaensis* affected Mg concentrations in the roots (minus(-)0.07) more severely than in the leaves ((-)0.03). Root-knot nematodes mobilize plant nutrients to nematode-induced nurse cells at infection sites. Thus, roots are damaged and foliar nutrient deficiencies occur because nutrient partitioning has been disrupted.

NEMATODE ESOPHAGEAL GLAND CELLS: THE KEY TO UNDERSTANDING NEMATODE PARASITISM OF PLANTS. R. S. HUSSEY. Dept. of Plant Pathology, University of Georgia, Athens, GA 30602.

Plant-parasitic nematodes evolved three large and complex esophageal glands that produce secretions required for plant parasitism. Each gland is a single large transcriptionally active secretory cell with a cytoplasmic extension that ends in a storage ampulla connected to the esophageal lumen by an elaborate valve. Secretory proteins are synthesized in the nuclear region of the cell and packaged in Golgi-derived membrane-bounded granules that are transported along microtubules in the gland extensions to the ampulla. Association of neural processes with the gland extensions indicates that the regulated secretion of proteins is controlled by the nematode's nervous system. During secretion, the secretory proteins are rapidly released from the granules by exocytosis into the membranous end-sac of the valve. The proteins then enter the esophageal lumen to be secreted through a protrusible stylet into plant tissues. Gland cell morphology and secretory proteins are under dynamic developmental regulation during nematode parasitism of plants. An understanding of the nature of these secretions and their function in parasitism is now beginning to emerge.

CONTROL OF SOILBORNE DISEASES IN POTATO WITH SHANKED-IN METAM SODIUM. R. E. INGHAM (1) and P. B. Hamm (2). (1) Dept. of Botany and Plant Pathology; (2) Dept. of Botany and Plant Pathology and Hermiston Agriculture Research and Extension Center.

In order to maintain yields and quality, potato growers in the Pacific Northwest, U.S. must control Columbia root-knot nematode (*Meloidogyne chitwoodi*), stubby-root nematode (*Paratrichodorus allius*) the vector of tobacco rattle virus which causes corky ring spot (CRS) disease, and *Verticillium dahliae*, which contributes to early dying disease. These pathogens have been controlled by double fumigation with shank injection of 1,3-dichloropropene (1,3-D) at 187 liters/ha (which controls nematodes but not *V. dahliae*) plus metam sodium at 355 liters/ha applied via chemigation (which controls *V. dahliae* but not nematodes). Although double fumigation is effective, it is also expensive. Trials with the two products in combination at reduced rates (140 liters/ha 1,3-D; 280 liters/ha metam sodium) demonstrated that control could be maintained at a savings of \$250/ha to the grower. Additional studies revealed that shank injection of metam sodium at 15,

30 and 45 cm provided better nematode control than when applied by chemigation. However, when used alone, a higher rate (561 liters/ha) may be needed for control of *M. chitwoodi*, because more soil volume is treated by this method. A tank mix of metam sodium at 355 liters/ha plus ethoprop EC at 18.7 liters/ha injected at 15 and 30 cm provided excellent control of *M. chitwoodi* and CRS.

TREHALOSE ACCUMULATION AT SUB-LETHAL TEMPERATURES BY ENTOMOPATHOGENIC NEMATODES AND ITS ROLE IN SURVIVAL AT ENVIRONMENTAL EXTREMES. **G. B. JAGDALE (1) and P. S. Grewal (2).** (1) Department of Entomology; (2) department of Entomology.

Cold acclimation at sub-lethal temperatures leads to trehalose accumulation in entomopathogenic nematodes. We hypothesized that trehalose accumulation in nematodes is a general strategy to prepare for survival at environmental extremes. Therefore, we tested whether the nematodes will accumulate trehalose during acclimation at sub-lethal warm and cold temperatures and whether the accumulated trehalose correlates with enhanced desiccation, heat, and freezing tolerance. Three species, *Steinernema carpocapsae*, *S. feltiae*, and *S. riobrave* were acclimated at 35 and 5°C for 1 and 4 days, respectively; their trehalose contents measured. Survival of acclimated- and non-acclimated nematodes at -20 and 40°C, and in 25% glycerol was compared. *S. riobrave* and *S. carpocapsae* accumulated high trehalose at 35°C, and *S. feltiae* at 5°C. Heat tolerance in acclimated *S. carpocapsae* and *S. feltiae* was high, but unaffected in acclimated *S. riobrave*. Freezing tolerance in acclimated *S. carpocapsae* and *S. riobrave* was increased, whereas in acclimated *S. feltiae* it was unaffected. Heat acclimated *S. carpocapsae* and cold acclimated *S. riobrave* showed the highest desiccation survival at 5°C. Overall, the accumulated trehalose levels appear to enhance desiccation, freezing and heat tolerance of entomopathogenic nematodes.

SUPPRESSED REPRODUCTION OF *GLOBODERA TABACUM SOLANACEARUM* ON DISEASE-RESISTANT CULTIVARS OF FLUE-CURED TOBACCO. **C. S. JOHNSON.** Southern Piedmont Agricultural Research and Extension Center, and Department of Plant Pathology, Physiology, and Weed Science, Virginia Polytechnic Institute and State University, Blackstone, VA 23824.

Globodera tabacum solanacearum (tobacco cyst nematode - TCN) is one of the most serious disease problems of flue-cured tobacco in Virginia. Efforts to develop TCN-resistant cultivars have not resulted in any commercially acceptable cultivars. However, suppressed TCN reproduction has been noted in tobacco cultivars developed for resistance to other diseases. Resistance to *G. t. solanacearum* has been linked with resistance to *Pseudomonas syringae* pv. *tabaci* (wildfire). Suppressed nematode reproduction was also discovered in the flue-cured tobacco cultivar 'Coker 371-Gold', developed for resistance to *Phytophthora parasitica* var. *nicotianae* (black shank). Five field experiments conducted in 1998-2000 have identified six additional black shank resistant cultivars (NC 71, NC 72, NC 297, RG H51, SP 168, and SP 179) that suppress TCN reproduction. Planting these cultivars should enable tobacco producers to reduce nematicide use and increase economic returns.

CHARACTERIZATION OF A MUTANT INVOLVED IN *MI-1*-MEDIATED RESISTANCE TO ROOT-KNOT NEMATODES IN TOMATO. **I. KALOSHIAN and O. Martinez de Ilarduya.** Department of Nematology, University of California, Riverside, CA 92521.

In tomato, resistance to three species of root-knot nematodes (RKNs) (*Meloidogyne* spp.) and to the potato aphid (*Macrosiphum euphorbiae*) is conferred by *Mi-1*. We are using a genetic approach to dissect *Mi-1*-mediated resistance pathway to RKNs. Using a fast neutron irradiated tomato population, we have isolated a mutant, *rme1* (for resistance to *Meloidogyne*) that showed complete susceptibility to *M. javanica*. Molecular and genetic data confirmed that *rme1* mutant plants had a single recessive mutation in a locus different from *Mi-1*. In addition to nematode susceptibility, this mutation also abolished aphid resistance. To assess whether *rme1* plants had increased susceptibility to virulent pathogens, the growth of *Pseudomonas syringae* pv. *tomato* strain DC3000 was monitored and found to be at similar levels in both wild type parent and *rme1* mutant plants. A similar experiment is currently undergoing with *Mi-1* virulent RKN. As a step towards cloning this locus, we have generated a segregating population and are in the process of identifying linked markers to map this gene.

EFFECT OF SUB-LETHAL DOSES OF SYSTEMIC NEMATICIDES ON ROOT-KNOT NEMATODE ON TOMATO. **A. A. KHAN.** Dept. Botany, Aligarh Muslim University, Aligarh, UP, India 202002.

Systemic nematicides, carbofuran and aldicarb gave promising effects on plant growth and root-knot development at sub-lethal doses of 10 ppm concentration for 6 hours of exposure. The plant growth of tomato and subsequent development of gall index and egg mass index was enhanced and reduced respectively when both plant and nematode were treated with each nematicide separately than that of untreated plant and nematode.

SPECIES OF PREDATORY SOIL NEMATODES (MONONCHIDA) FROM JAPAN. **Z. KHAN and M. Araki.** Nat'l Inst. of Agro-Envir. Sciences, Tsukuba, Ibaraki, Japan.

A study was undertaken to find out species diversity of soil nematodes in Japan. A new and two known species of predatory nematodes belonging to the order Mononchida were isolated from the soil samples collected from a deciduous forest of Hinokuma mountain, Saga prefecture, Japan. TAF-fixed nematodes were processed to glycerin-mounted slides. Specimens were observed under optical microscope and measured with the help of a drawing tube attachment. A new species of *Iotonchus* is 3.1-3.5 mm long; a = 44-50; c = 7.6-11.0; V = 58-62; buccal cavity 64-71 × 38-46 microns (um); spicules 128-143 um long; ventromedian supplements 10-13. *Clarkus sheri* (Mulvey, 1967) Jairajpuri, 1970 is 1.9-2.0 mm long; a = 32-35; c = 18-21; V = 63-64; buccal cavity 41-43 × 22-23 um. Japanese specimens are very similar to those described by Mulvey but have slightly longer buccal cavity. *Mylonchulus polonicus* (Stefanski, 1915) Cobb, 1917 is 1.6-2.0 mm long; a = 33-41; c = 15.6-18.0; V = 62-66; buccal cavity 32-36 × 19-23 um. Morphology and measurements of present population of this species confirms well with original description. *C. sheri* and *M. polonicus* are reported herein for the first time from Japan.

UTILIZATION OF VARIOUS CROPPING SEQUENCES FOR CONTROL OF SOYBEAN NEMATODES. P. S. KING and R. Rodriguez-Kabana. Dept. Entomology and Plant Pathology, Auburn University, Alabama 36849-5409.

A four year field study was initiated to determine the efficacy of pearl millet [PM], velvetbean [VB], sorghum [SG], corn [CR] and cotton [CT] used in various rotational sequences with soybean [SB] for control of plant-parasitic nematodes. The field had been under cotton monoculture the preceding ten years and the soil was infested with *Meloidogyne* spp., *Pratylenchus* spp., *Paratrichodorus minor*, *Tylenchorhynchus claytoni* and *Hoplolaimus galeatus*. Soybean monoculture supported all nematode pathogens. Nematodes were not suppressed in response to [SB-SB-PM-SB] or [SB-SB-SG-SB]. Moderate suppression of nematodes was noted in response to [SB-SB-CT-SB]. Soybean yields were not significantly increased in response to any of these cropping systems. The most effective cropping system for suppressing nematodes was [VB-VB-VB-SB], however, no significant soybean yield increases were noted in this system. The only cropping systems evaluated in the study that showed significant suppression of nematodes as well as some significant increases in soybean yields were [VB-VB-CR-SB] and [VB-VB-PM-SB].

MECHANISMS BY WHICH TYLENCHULUS SEMIPENETRANS MAY MITIGATE VIRULENCE OF PHYTOPHTHORA NICOTIANAE TO CITRUS SEEDLINGS. F. E. KORA (1), L. W. Duncan, and J. H. Graham. (1) University of Florida, Citrus Research and Education Center, Lake Alfred, Florida, 33850, U.S.A.

Efficacy of nematicides to manage *T. semipenetrans* in field experiments was inversely related to population density of *P. nicotianae*. The results imply competition between these two parasites of the citrus fibrous root cortex. We tested this hypothesis and found that infection of roots by *T. semipenetrans* reduces subsequent infection and damage to citrus seedlings by *P. nicotianae*. Surveys and bioassays revealed that 1) microorganisms in the citrus rhizosphere occur in greater numbers on nematode-infected roots than on non-infected roots, 2) some microorganisms mitigate the virulence of *P. nicotianae* to citrus seedlings, 3) eggs of the citrus nematode suppress the growth of *P. nicotianae* in vitro. Our research has demonstrated an antagonistic effect of a plant parasitic nematode on a fungal plant pathogen, and reveals potential mechanisms involving direct inhibition of the fungus by the nematode and indirect mitigation of fungal virulence mediated by complex microbial interactions in the citrus rhizosphere.

TRITROPHIC INTERACTIONS BETWEEN PERENNIAL RYEGRASS, BLACK CUTWORMS, AND AN ENTOMOPATHOGENIC NEMATODE. B. A. KUNKEL and P. S. Grewal. The Ohio State University.

Some grasses form symbioses with *Neotyphodium* fungi. These endophytic fungi enhance plant growth, drought tolerance, and resistance against herbivory. Many insect species are susceptible to the alkaloids produced by plants containing the endophytes. However, black cutworm, *Agrotis ipsilon*, appears to be an exception. As some insects can transform plant alkaloids into novel compounds, we hypothesize that the black cutworm may use them to increase its defense against an entomopathogenic nematode. Cutworms, fed on endophytic or non-endophytic perennial ryegrass (*Lolium perenne*) grass for 1 week, were exposed to the entomopathogenic nematode, *Steinernema carpocapsae*. Mortality was recorded at 16 h post exposure and every 4 h thereafter for 48 h. Our results support the hypothesis that cutworms feeding on plants with high (>90%) incidence of *Neotyphodium* endophytes are less susceptible to entomopathogenic nematodes than plants with no or low incidence of endophyte (0-60%). This acquired tolerance/resistance may be due to alteration of endophyte alkaloids by the developing larvae to defensive compounds.

SITE SPECIFIC MANAGEMENT IN PLANT PATHOLOGY. J. E. Kurle. Dept. of Plant Pathology, University of Minnesota, St. Paul, MN 55108.

Site-specific management may provide powerful tools for the plant pathologist. Currently site-specific management practices emphasize assessment of relatively stable factors such as soil pH or soil fertility. Often these factors do not explain yield variation in intensively mapped fields. By broadening understanding of seasonally or spatially variable biotic factors, such as plant pathogens, the plant pathologist can increase the effectiveness of site-specific management practices. GIS,

GPS, yield monitors, and remote sensing technologies are also powerful tools for analysis and description of the effects of plant pathogens. The spatial information they provide combined with variable rate technologies can become a tool for managing plant disease. For instance, maps of nematode population density have been used to guide nematicide application and models of site topography or microclimate have been used to prescribe fungicide use. This predictive capability linked to variable rate planters or sprayers enables manipulation of management factors such as variety, planting rate or the application of pesticides to control plant diseases.

CLONING AND ANALYSIS OF ROOT-KNOT NEMATODE ESOPHAGEAL GLAND GENES. K. N. LAMBERT. Department of Crop Sciences, University of Illinois, Urbana IL, 61801.

To gain insight into how plant pathogenic nematodes parasitize plants, we have been isolating and characterizing nematode genes that are expressed in the esophageal region of the root-knot nematode, *Meloidogyne javanica*. To date, we have isolated over 30 genes that appear to be expressed in esophageal glands of these nematodes. The deduced protein sequences for all these genes appear to have signal peptides, indicating that these nematode genes could encode secreted proteins. The gland genes fall into several classes; some appear to encode secreted proteins that may be injected into plant cells, others may be secreted around plant cells, while still others appear to be retained within the nematode. In this paper we will summarize what we have learned about a nematode chorismate mutase, a pectate lyase-like protein, and several neuropeptides. We will also discuss other recently cloned gland genes and their potential roles in nematode parasitism.

MONITORING HEMLOCK FOREST HEALTH USING SATELLITE CHANGE DETECTION TECHNIQUES. R. G. LATHROP (1) and D. D. Royle (1). (1) Center for Remote Sensing & Spatial Analysis, Rutgers University, New Brunswick, NJ 08901.

The eastern hemlock (*Tsuga canadensis*) has been declining in health and vigor in eastern North America due to infestation by an introduced, sap-feeding insect, the hemlock woolly adelgid (HWA) (*Adelges tsugae*). HWA defoliation is characterized as a slow loss of needles and a reduction in new growth, resulting in a gradual thinning of the hemlock forest canopy. In order to monitor the spatial extent and severity of HWA-associated hemlock decline, we have investigated the application of various forms of remotely sensed change detection. Using medium scale satellite remote sensing systems, such as the Landsat Thematic Mapper, we have demonstrated that it is feasible to quantify the decline in hemlock across spatially heterogeneous forested landscapes. As defoliation occurs in the forest, the biomass of green foliage is reduced resulting in decreased near infrared and increased red reflectance, a change captured in the remotely sensed vegetation index. By differencing pre-infestation vs. post-infestation vegetation index images of near infrared to visible red wavebands, it is possible to rank the decline into five broad classes: healthy, light, moderate, severe and dead. Using a time series of vegetation index images, we are investigating temporal and spatial patterns in the infestation and decline process over the past decade. Based on the success of this methodology, we are now expanding our study to monitor hemlock decline across the entire Mid-Atlantic U.S.A. region.

FIELD RESPONSE OF MID-SOUTH COTTON VARIETIES TO THE RENIFORM NEMATODE. G. W. LAWRENCE (1), K. S. McLean (2), H. K. Lee (1), and W. Price (1). (1) Department of Entomology and Plant Pathology, Mississippi State University, Mississippi State, MS 39762 and (2) Department of Entomology and Plant Pathology, Auburn University, Auburn, AL 36849.

Twenty-two mid-south cotton varieties were evaluated for resistance and yield to the reniform nematode (*Rotylenchulus reniformis*). The test was conducted in a field located in the Mississippi Delta at Glen Allan, Mississippi which was naturally infested with the reniform nematode. Each variety was planted with and without the nematicide aldicarb (0.59 kg a.i./ha). The varieties that did not receive aldicarb were treated with disulfoton (0.85 kg a.i./ha) for early season insect control. A reniform population density of 9,850 nematodes / 500cm² across all varieties was recovered at planting. Each cotton variety varied in their response to the aldicarb applications. Seed cotton yields were improved 54.4 to 1,281.3 kg / ha with the addition of aldicarb from Stoneville x9903 and Stoneville 6MO45, respectively. Four varieties resulted in lower yields with the addition of aldicarb. None of the cotton varieties included in this test showed resistance to the reniform nematode.

HORIZONTAL AND VERTICAL DISTRIBUTION OF THE RENIFORM NEMATODE. H. K. LEE (1) and G. W. Lawrence (2). (1) Department of Entomology and Plant Pathology, Mississippi State University, Mississippi State, MS 39762.

The horizontal and vertical distribution of the reniform nematode (*Rotylenchulus reniformis*) was determined in a 20 acre field continuously cultured with cotton located in the Mississippi Delta at Glen Allen, Mississippi. The cotton field was mapped into twenty points on 0.52 hectare grids using a Global Positioning System (GPS). A single soil core, dimensions 5.08 cm diameter × 121.9 cm deep, was collected from each of the grid intersections 25 days after planting using a Model 4804 Concord Soil Sampler. Each core was divided into 8 depths: 0-15 cm, 16-30 cm, 31-45 cm, 46-60 cm, 61-75 cm,

76-90 cm, 91-105 cm, and 106-120 cm. Reniform nematodes were found at each of the twenty sample points. The number of nematodes recovered from each of the twenty points ranged for 2,996 to 11,870 nematodes per 100 cm³ of soil. Reniform numbers at 0-15 cm, 16-30 cm, 31-45 cm, 46-60 cm, 61-75 cm, 76-90 cm, 91-105 cm, and 106-120 cm depths averaged 899, 1,030, 1,198, 1,420, 1,258, 791, 765, and 370 nematodes per 100 cm³ of soil, respectively. The highest numbers of nematodes were recovered from the 31-45 cm depth.

EFFECT OF THE *RHG1* GENE ON LIFE CYCLE OF THE SOYBEAN CYST NEMATODE. **Y. H. LI (1), S. Y. Chen (1), N. D. Young (1), and J. H. Orf (2).** (1) Department of Plant Pathology; (2) Department of Agronomy and Plant Genetics, University of Minnesota, St. Paul, MN 55108.

Soybean resistance to the soybean cyst nematode (SCN) has been documented as a complex trait and a small number of minor genes involved in resistance. The *rhg 1* gene has been shown to be responsible for 50% or more of the resistance phenotype. Near-isogenic lines (NILs) differing in the genomic regions surrounding the *rhg 1* locus allowed us to examine the *rhg 1* gene with regard to its function. Especially, we analyzed the influence of *rhg 1* on nematode hatching, penetration, development, and reproduction. No difference in the influence of root exudates on SCN hatch was observed between resistant and susceptible NILs under both growth-room and in vitro conditions. An inbred line of SCN race 3 that was completely unable to reproduce on the race differential lines Peking, Pickett, PI88788, and PI90763 could reproduce on both NILs, although the lines containing *rhg 1* supported fewer nematodes than lines without the *rhg 1* gene. The *rhg 1* gene reduced the number of eggs produced per female. Experiments are underway to determine the effect of *rhg 1* gene on SCN penetration and development.

BURKHOLDERIA CEPACIA COMPLEX AS HUMAN PATHOGENS. **J. J. LiPuma.** Dept. Pediatrics, University of Michigan Medical School, Ann Arbor, MI 48109.

Although generally nonpathogenic for healthy persons, *Burkholderia cepacia* has long been recognized as capable of causing sporadic human infection. Individuals with chronic granulomatous disease (CGD), a primary immunodeficiency marked by dysfunctional white blood cells, are particularly susceptible to infection. During the late 1970s *B. cepacia* also emerged as a significant pathogen in persons with cystic fibrosis (CF), a much more common inherited disease. Inherent broad-spectrum resistance renders most infections refractory to effective antimicrobial therapy; outcomes vary from chronic infection to rapidly fatal illness. Recent studies indicate that although all species of the *B. cepacia* complex can infect persons with CF, the distribution of species in this patient population is quite disproportionate, suggesting a differential capacity to cause human infection. Studies employing isolate genotyping have demonstrated the existence of some clonal lineages that seem to have an enhanced capacity for transmission and persistence within patient groups. Ongoing studies seek to identify bacterial virulence factors and to better define host-pathogen interactions.

APPLIED GENOMICS IN NEMATODE DISCOVERY: AN INDUSTRY PERSPECTIVE. **L. X. LIU.** Cambria Biosciences, 2 Preston Court, Bedford, MA 01730.

Now that we are in the era of total genomes, what constitutes a desirable molecular target for intervention to control plant and animal parasitic nematodes? Although the unmet needs in crop protection are very different from those in animal health, economic considerations for both markets dictate that any new anti-nematode molecules be not only potent and safe, but also effective against a broad spectrum of parasitic species affecting many different hosts. This argument suggests that we should target shared processes in nematode physiology and behavior, rather than parasitism functions that are particular to a specific, highly evolved parasite-host association. The free-living nematode *Caenorhabditis elegans* provides a wealth of information and tools for nematode biology, including complete delineation of its development, anatomy, neuroanatomy, and genome, and well-established techniques for systematic forward and reverse genetics. Furthermore, *C. elegans* is readily adaptable to microplate-based liquid handling for use in high-throughput chemical screens. I will discuss the power of *C. elegans*, using specific examples, for "gene-to-screen" discovery of candidate nematicides.

NEMATODE HOSTS OF THE FUNGUS *HIRSUTELLA MINNESOTENSIS*. **S. F. LIU and S. Y. Chen.** University of Minnesota Southern Research and Outreach.

Hirsutella minnesotensis was first isolated from the second-stage juveniles of *Heterodera glycines*. The objective of this study was to determine whether the fungus can parasitize various nematodes. Four isolates (WA23-1, FA2-1, MA13-1 and Hm134) of the fungus were tested on 15 nematode species including plant-parasitic nematodes: *Belonolaimus* sp., *Criconemella* sp., *Heterodera glycines*, *Hoplolaimus* sp., *Meloidogyne arenaria*, *Meloidogyne hapla*, *Meloidogyne incognita*, *Meloidogyne javanica*, *Rotylenchulus reniformis* and *Scutellonema* sp.; entomophilic nematodes: *Steinernema glaseri* and *Heterorhabditis bacteriophora*; and fungal-feeding nematodes: *Aphelenchus* sp. and *Aphelenchoides* sp. The nematodes were exposed to the fungal cultures on corn meal agar for 3, 7, and 14 days. Percentages of nematodes with attached spore(s) or filled with mycelium were recorded. When mycelium development in nematode body was observed, the

nematode was considered parasitized. Parasitism by the fungus was observed at day 3 for all of the nematodes. The results showed that the host range of *H. minnesotensis* is very wide.

SPORULATION OF *HIRSUTELLA MINNESOTENSIS* GROWING FROM SECOND-STAGE JUVENILES OF *HE-TERODERA GLYCINES*. S. F. LIU and S. Y. Chen. University of Minnesota, Southern Research and Outreach Center, Waseca, MN.

Hirsutella minnesotensis is a new parasite of the second-stage juveniles (J2) of *Heterodera glycines*. The objective of this study was to determine when it sporulates and how many spores it produces for each colonized J2. Three isolates (FA2-1, WA23-1 and MA13-1) of the fungus were examined. J2 were exposed to fungal cultures on corn grits for 2 days before they were transferred onto a circular area of a petri dish where a 1-cm-diam water agar disk had been removed and 0.1 ml of water had been added. The dishes were sealed with parafilm and maintained at 25° C. The number of spores produced by the fungus growing from each J2 was recorded at intervals of 1-4 days for 38 days after inoculation (DAI). Results showed FA2-1 and WA23-1 began sporulation at 7 DAI, and MA13-1 at 10 DAI. The highest numbers of spores on the mycelium per colonized J2 were 23 for FA2-1 at 12DAI, 35 for WA23-1 at 14 DAI, and 16 for MA13-1 also at 14 DAI. At 17 DAI, most spores germinated, but after 18 DAI the number of spores on the mycelium decreased quickly. At 38 DAI, no or only a few spores were observed on the mycelium.

ANALYSIS OF GENETIC VARIATION AMONG ISOLATES OF *MELOIDOGYNE HAPLA*. Q. LIU, C. Gleason, and V. M. Williamson. Department of Nematology, University of California, Davis 95616.

The northern root-knot nematode (*Meloidogyne hapla*) causes serious damage in dicotyledonous crops. Isolates of this species can differ in host range. Using AFLP (amplified fragment length polymorphism) markers, we have identified DNA polymorphisms among isolates, some of which differ in ability to reproduce on *Solanum bulbocastanum*. We are investigating isolate specific markers in order to follow the segregation of phenotypes. We have also been inbreeding these isolates by single eggmass transfer for several generations.

BIOLOGICAL CONTROL EFFECTIVITY OF *RHIZOBIUM ETLI* G12 TOWARDS SEDENTARY AND MIGRATORY NEMATODES ON VARIOUS HOST PLANTS. M. Mahdy, J. HALLMANN, and R. A. Sikora. Institut für Pflanzenkrankheiten, Nussallee 9, D-53115 Bonn, Germany.

Rhizobium etli G12 is a biological control agent with antagonistic activity against plant parasitic nematodes. The main control mechanism is considered to be induced systemic resistance in the host plant. The control potential of *R. etli* G12 was tested towards a broad spectrum of plant parasitic nematodes and host plants under greenhouse conditions. When applied as a soil drench, *R. etli* G12 significantly reduced the number of galls caused by *Meloidogyne incognita* on tomato (43%), cotton (41%), pepper (39%), soybean (39%), cucumber (34%) and potato (30%). The number of new cysts produced was significantly reduced for *Heterodera schachtii* on sugar beet and *Globodera pallida* on potato. *R. etli* G12 did not affect *Pratylenchus zaeae* densities on corn. Reduction in nematode infestation was generally associated with increased plant growth, although not always significantly.

CHEMICAL MANAGEMENT OF NEMATODES IN LOUISIANA: FIELD AND MICROPLOT TRIALS WITH COTTON, SOYBEAN, RICE AND ASSORTED VEGETABLE SPECIES. E. C. MC GAWLEY (1) and C. Overstreet (2). Research (1) and Extension (2) Nematologists, LSU Agricultural Center, Baton Rouge, LA 70803.

Field trials were conducted to evaluate Telone (45.3 and 90.6 kg a.i./ha) and two experimental compounds, Agri 50 and 60 (10 GPA diluted 1:100), on soybeans and cotton. On both crops, application of Telone and Agri 50 reduced the numbers of reniform nematodes at midseason and at harvest. Telone produced increases (14 and 21%) in soybean yield and Agri 50 and 60 produced numerical (12% and 14%, respectively), but non-significant yield responses. Weights of seed cotton were increased by Telone and Agri 50. Seed cotton from untreated plots averaged 2.5 kg per 80' row. Yields from Telone and Agri 50-treated plots averaged 6.1 and 5.0 kg. Microplot trials included tomato, cotton, and rice. Inoculated tomato plants had gall indices which averaged 4.3 and Agri 50 treated plants averaged 0.5. On cotton, both Agri 50 and 60 reduced numbers of reniform nematodes. Ring and stunt nematodes were employed in the study with rice. Compared to the control, Agri 50 reduced the total nematode at harvest from 381,590 individuals per microplot to an average of only 42,020 individuals.

FIELD RESPONSE OF TRANSGENIC AND NON-TRANSGENIC COTTON VARIETIES TO THE RENIFORM NEMATODE. K. S. MCLEAN (1), G. W. Lawrence (2), W. S. Gazaway (1), A. J. Palmateer (1), and J. R. Akridge (1). (1) Department of Entomology and Plant Pathology, Auburn University, Auburn, AL 36849; (2) Department of Entomology and Plant Pathology, Mississippi State University, Mississippi State, MS 39762.

Eighteen transgenic and non-transgenic cotton varieties were evaluated for resistance and yield to the reniform nematode (*Rotylenchulus reniformis*). The test was conducted in a field located in Huxford, Alabama which was naturally infested

with the reniform nematode. Each variety was planted with and without the nematicide aldicarb at 0.59 kg a.i./ha. The varieties that did not receive aldicarb were treated with disufoton at 0.85 kg a.i./ha for early season insect control. A reniform population density of 3,000 nematodes /150 cm³ across all varieties was recovered at planting. Each cotton variety varied in their response to the aldicarb applications. Seed cotton yields were improved 5.58 to 225.19 kg / ha with the addition of aldicarb from Delta Pearl and Stoneville 4892BR, respectively. Nine varieties resulted in lower yields with the addition of aldicarb. None of the cotton varieties included in this test showed resistance to the reniform nematode.

ENDOPARASITIC FUNGAL COLONIST OF *ROTYLENCHULUS RENIFORMIS*. K. S. MCLEAN (1), G. W. Lawrence (2), A. J. Palmateer (1), and G. Morgan-Jones (1). Departments of Entomology & Plant Pathology.

The reniform nematode (*Rotylenchulus reniformis*) was collected from fields continuous cropped in cotton and examined for fungal antagonists. Mature females and vermiform stages of the reniform nematode were surface sterilized and plated on potato dextrose agar. *Acremonium* sp. and *Arthrographis* sp. occurred in the highest frequency followed by *Chaetomium aureum*, *Fusarium equiseti*, and *Pseudorobillarda* sp. Of the total reniform population extracted from the soil, approximately 0.015% were visibly colonized by fungi. In greenhouse pathogenicity tests, *Acremonium* sp., *Pseudorobillarda* sp., *Fusarium equiseti*, significantly reduced reniform nematode population development on cotton. The reniform reproductive factor (Rf = initial population/final population) ranged from a low of 1.22 to a high of 5.19 for *Fusarium equiseti* and the untreated control, respectively. Preliminary results from this study demonstrate that fungi do exist in nature that are capable of parasitizing the reniform nematode.

EMBRACING THE EMERGING PRECISION AGRICULTURE TECHNOLOGIES-SYMPIOSIUM INTRODUCTION. H. Melakeberhan. Department of Entomology, Michigan State University, East Lansing, MI 48824.

Precision agriculture (PA), an information revolution utilizing global positioning (GPS) and geographic information (GIS) systems and remote sensing (RS) for better decision making, is gaining momentum to site-specifically manage (SSM) crop yield-limiting biotic and abiotic factors and their interactions in a wide range of production systems. Characterizing the nature of the problem(s) and public education are among the challenges that scientists, producers and industry face when adapting PA technologies. In order to apply SSM, spatiotemporal characteristics of the problem(s) need to be determined and variations within a field demonstrated. Spatiotemporal characteristics of a given pathogen or pest problem may be known, but it may not be the only or primary cause of the problem. Hence, the exact cause-and-effect relationships need to be established by incorporating GIS, GPS and RS generated data and possible interactions as well. Exploiting the potential of PA technologies in sustainable ways depends on whether or not we first ask "Are we doing the right thing?" (strategic) as opposed to "Are we doing it right?" (tactical).

RESISTANCE TO SOYBEAN CYST NEMATODE: BIGENIC INHERITANCE AND CLUSTERING WITH RESISTANCE TO *FUSARIUM SOLANI*. K. MEKSEM (1), A. Jamai (1), E. Ruben (1), K. Zobrist (1), K. Triwitakorn (1), P. Arelli (2), and D. A. Lightfoot (1). (1) So. Illinois Univ., Carbondale, IL 62901; (2) Univ. of Missouri, Columbia, MO 65211.

A chromosomal region on molecular linkage group G in the soybean cultivar Forrest underlies co-inheritance of field resistance to sudden-death syndrome caused by *F. solani* f.sp. *glycines* and soybean cyst nematode (SCN) race 3. A high-density genetic map was constructed around the *Rhg1* and *Rhg4* loci that underlie SCN resistance. Seven recombinants were positioned in a 150-kbp interval containing *Rhg1*. Candidate gene sequences for *Rhg1* and *Rhg4* were isolated. A leucine rich repeat sequence (LRR), a trans-membrane spanning domain, and a kinase domain were isolated. The identified gene shows high homology to the *Xa21* rice disease resistance gene and to an *Arabidopsis* receptor-like kinase. The Forrest allele contains 76 leucines in 13 extra-cellular GXXP motifs and a kinase on the intracellular domain. A gene closely homologous to *Rhg1* is found in the Forrest *Rhg4* interval. This gene duplication and the large number of LRRs imply *Rhg* genes function by dimerization, which may explain variation in host-pathogen recognition.

THE FIRST RECORD OF THE DISSEMINATION OF *DITYLENCHUS DIPSACI* BY SEED IN TURKEY. S. MENNAN (1) and O. Ecevit (1). (1) 19 May University, Agricultural Faculty, Plant Protection Department, Samsun, Turkey.

It was revealed before that the percentage of *Ditylenchus dipsaci* infected onion fields in Suluova, Amasya province (Turkey) was found to be 54%. To reveal the contamination sources of *D. dipsaci* in Suluova, onion seeds, irrigation water and infested plant material from previous year were analysed and it was found all sources affected spreading the pest from contaminated to clean areas. It was found that, *D. dipsaci* can be transmitted by using all of those. It was a new record for Turkey that contamination of *D. dipsaci* can be occurred by onion seeds. The percentage of *D. dipsaci* contaminated onion seeds collected from Suluova was found to be 26%. The seeds probably contaminated by the fact that *D. dipsaci* moves up in plants as a shown with samples taken in monthly surveys from different parts of onion plants. As a result of the studies, the onion farmers were advised to use clean onion seeds in order to protect the damage of *D. dipsaci*.

DEVELOPMENT OF MOLECULAR MARKERS FOR RESISTANCE TO CLOVER ROOT-KNOT NEMATODE. **C. F. MERCER, B. Barrett, A. G. Griffiths, D. R. Woodfield, and K. Moore.** AgResearch Grasslands, Private Bag 11008, Palmerston North, New Zealand.

Infections of the clover root-knot nematode (CRKN), *Meloidogyne trifoliophila*, reduce forage yield. A recurrent selection program in white clover, *T. repens*, has developed genotypes exhibiting a partial resistance phenotype. A mapping effort has been initiated to develop a suite of molecular markers for use in marker-assisted selection (MAS). We used bulked segregant analysis (BSA) to identify putative markers segregating with the resistant phenotype which are being confirmed among F1 progeny. Another potential source of resistance to CRKN is Kenya white clover *T. semipilosum*, a diploid relative of white clover which has some immune genotypes. Molecular markers are being used to tag the resistance in *T. semipilosum* using BSA among F1 progeny. These markers may then be used, not only for MAS within *T. semipilosum*, but also for marker-assisted introgression of the immunity from *T. semipilosum* into white clover. Markers developed in *T. semipilosum* also offer a starting point for physical mapping and positional cloning of the gene(s) conferring the immune phenotype.

IDENTIFICATION OF NEMATODE-ANTAGONISTIC COMPOUNDS FROM FUNGI. **S. L. F. MEYER, J. K. Nitao, and D. J. Chitwood.** USDA ARS, Nematology Laboratory, Beltsville, MD 20705.

Fungi that produce compounds antagonistic to plant-parasitic nematodes can be used as biocontrol agents or as sources for the active compounds. Fungus isolates (ca. 250) were tested for production of compounds active against root-knot nematode (*Meloidogyne incognita*) and soybean cyst nematode (*Heterodera glycines*). Fungi were cultured in potato dextrose broth (PDB), and biomass was removed by centrifugation and filtration. Nematode egg hatch in the filtrates was 2% to 224% of hatch in PDB controls. Juvenile mobility was inhibited by few of the filtrates. Active compounds were identified from two fungi. The compounds from *Fusarium equiseti* were the trichothecenes 4,15-diacetylivalenol and diacetoxyscirpenol; trichothecenes are toxic to a number of organisms. The active agent from *Chaetomium globosum* was flavipin. These three compounds have previously been isolated from fungi, but this is their first reported activity against plant-parasitic nematodes. Flavipin solutions were applied at 0, 30, 60, and 120 micrograms/ml to *Cucumis melo* plants in greenhouse studies with *M. incognita*. The treatments did not suppress gall formation nor egg and J2 numbers.

USING SOIL ELECTRICAL CONDUCTIVITY TO PREDICT THE DISTRIBUTION OF COTTON NEMATODES. **J. D. MUELLER (1), A. Khalilian (2), F. J. Wolak (2), and Y. Han (2).** (1) Dept. Plant Pathology and Physiology, Clemson Univ.; (2) Agricultural and Biological Engineering Dept., Clemson Univ.

The limiting factor in developing variable rate nematicide applications is determining the distribution of the target nematode species in a field. Intensive grid sampling is cost-prohibitive. Use of soil electrical conductivity (SEC) to measure soil texture and predict the distribution of three nematode species was very successful in a 10-acre loamy sand field in Barnwell County, SC. SEC had a positive correlation (0.92) with percentage clay and a negative correlation (0.91) with percentage sand. At-planting and harvest soil samples showed distinct distribution patterns for three nematode species when SEC (mS/M) was divided into four ranges. Recovery of *Hoplolaimus columbus* decreased as SEC increased. An increase in soil clay content of 9% resulted in a 57% reduction in *H. columbus* density. This technology may allow estimation of the distribution by soil type of selected nematode species and variable rate nematicide application to those sites in a cost-effective manner.

STATUS OF *CAMPYDORA* (NEMATODA: CAMPYDORINA). **P. G. MULLIN (1), A. L. Szalanski (1), T. S. Harris (1), and T. O. Powers (1).** (1) Department of Plant Pathology, University of Nebraska-Lincoln.

The systematic position of *Campydora* Cobb, 1920, which possesses many unique morphological features (especially in esophageal structure and stomatal armature) has long been a matter of controversy. Thorne (1939) remarked that the "position of the Campydorinae" (containing only *Campydora*) was questionable. Jairajpuri (1983) reviewed the morphology of *C. demonstrans* Cobb, 1920 (sole species of *Campydora*) and concluded that the species warranted placement as the sole member of a monotypic suborder, Campydorina, in the order Dorylaimida. This placement of *Campydora* was unaltered in Jairajpuri and Ahmad's 1992 text on the Dorylaimida. Phylogenetic analysis of DNA sequences generated from the 18s small subunit ribosomal DNA, under both maximum parsimony and maximum likelihood, reveals that *Campydora* shares more recent common ancestry with genera such as *Alaimus*, *Amphidelus* (Alaimida), *Tripyla* and *Ironus* (Tripylida) than with any members of Dorylaimida or Triplonchida. Taxonomic placement of the genus *Campydora*, and the identity of its closest living relatives, is in need of further investigation.

NEMATODES IN WETLAND SOILS OF NORTH CAROLINA. **D. A. NEHER (1), M. E. Barbercheck (2), O. Anas (2), and S. El-Allaf (2).** (1) University of Toledo; (2) North Carolina State University.

Nematodes were identified to family from 500-ml samples collected 13 times in 1994-95 from an ephemeral saturated, pine wetland in each Jones (J), Lincoln (L), and Henderson (H) counties. Soils were hydric, acidic (mean pH 3.32, 4.92,

and 4.69), and rich in organic matter (mean 41.6, 1.9, and 8.4%). Total abundances were greatest in spring and fall months, dominated mostly by bacterivores and plant-parasites. Most common bacterivores included Cephalobidae, Rhabditidae, *Prismatolaimus*, Alaimidae and Plectidae. Abundances of plant-parasites were greatest in Tylenchidae, Hoplolaimidae, Criconematidae and Tylenchulidae. Most numerous among fungivores were *Aphelenchus*, Diphtherophoridae and *Aphelenchoides*. Dorylaimidae and Mylonchulidae were the most abundant omnivores and predators, respectively. Site J differed from the other two sites by containing an undescribed Bastianidae genus, an undescribed *Prismatolaimus* species, and presence of *Ogma* (Criconematidae), *Ecphyadophora* (Tylenchidae) and *Trophotylenchus* (Tylenchulidae). This is one of the first reports of nematode communities in wetland soils.

A REVISED CLASSIFICATION SCHEME FOR GENETICALLY DIVERSE POPULATIONS OF *HETERODERA GLYCINES*. T. L. NIBLACK (1), R. D. Riggs (2), P. R. Arelli (1), G. R. Noel (3), C. H. Opperman (4), J. H. Orf (5), D. P. Schmitt (6), J. G. Shannon (1), and G. L. Tylka (7). (1) U. Missouri; (2) U. Arkansas; (3) USDA-ARS; (4) N. Carolina St. U.; (5) U. Minnesota; (6) U. Hawaii; (7) Iowa St. U.

Field populations of *H. glycines* are given race designations based on the percentage of females that develop on four differentials compared with Lee 64. This system provided critical information to extension and breeding programs when only Peking, PI 88788, and PI 90763 had been used to breed for *H. glycines* resistance. Cultivars or germplasm lines with four alternative resistance sources have since been registered. These lines, PIs 437654, 209332, 89772, and Cloud, differentiate *H. glycines* populations in discriminant function analysis. We propose an updated system that 1) includes four new differentials, 2) excludes Pickett, and 3) changes the convention of naming populations to allow easier addition of new, properly registered differentials.

SELECTION AND REPRODUCTION OF *HETERODERA GLYCINES* ON RESISTANT SOYBEAN GERMPLASM. G. R. NOEL (1,2), S. J. Bauer (2), M. S. Assunção (3), and N. Atibalentja (2). (1) USDA, ARS; (2) Dept. of Crop Sciences, Univ. of Illinois, Urbana, IL 61801; and (3) EMBRAPA Goinia, GO, Brasil.

Populations of *H. glycines* races 1-6, 9, and 14 were selected for 2 years on soybean lines Cloud, Peking, PI88.788, PI89.772, PI907.63, and PI209.332. Each soybean line was inoculated with each of the selected populations to determine which resistant germplasm might be used in gene deployment to manage *H. glycines*. Of particular interest were selections on PI88.788, since it is the source of resistance for ca. 95% of resistant cultivars grown in the Midwest. Populations of each race selected on PI88.788 and then challenged with PI89.722 resulted in a female index (FI) = 0 or 1 except for race 4 for which FI = 19. Similar results were obtained with PI90.763 and Peking, except that FI for race 4 was greater on PI90.763 and less on Peking, than on PI89.772. Cloud and PI209.332 were similar for all populations selected on PI88.788 with FIs ranging from 12 to 68. When inoculated onto the source on which selected, a general resistance was observed except for Cloud. It was the only source of resistance for which selection resulted in an FI equal to susceptible 'Lee 74'.

USE OF GIS AND REMOTE SENSING TO DETECT THE PLANT STRESS CAUSED BY SOYBEAN CYST NEMATODE. F. W. NUTTER, Jr. (1), G. L. Tylka (1), J. Guan (1), A. J. D. Moreira (1), C. Marett (1), J. P. Basart (2), and C. S. Chong (2). (1) Dept. Plant Pathology, (2) Dept. of Electrical & Computer Engineering, Iowa State Univ., Ames, IA 50011.

Integrating remote sensing and geographic information systems (GIS) technologies into cropping system offers tremendous new opportunities to narrow yield gaps. In 2000, 995 2 × 3 m soybean quadrats infested with SCN were established and SCN population densities were determined. Ground and aerial remote sensing measurements were obtained every other week during the growing season. Soybean yield, protein and oil concentration were obtained. Geospatially referenced maps were generated to depict within-field variation in yield, protein, oil concentration, and SCN population densities. Quadrats with low SCN densities had a different temporal pattern in percentage reflectance (810 nm) compared to quadrats with high SCN densities. Percentage reflectance (810 nm) explained up to 90% of the variation in soybean yield and up to 52% of the variation in SCN population density. Remote sensing data obtained at the ground level explained up to 95% of the variation in aerial images.

ESTABLISHING A *PRUNUS* ROOTSTOCK EVALUATION SITE ON LAND WITH NO HISTORY OF SHORT LIFE OR PEACH PRODUCTION. A. P. NYCZEPIR, W. R. Okie, and T. G. Beckman. SE Fruit & Tree Nut Research Laboratory, USDA-ARS, Byron, GA 31008.

Peach tree short life (PTSL) is associated with the presence of ring nematode, *Mesocriconema xenoplax* (Mx), and poor management practices. Finding a non-commercial field site to evaluate rootstocks for PTSL resistance is increasingly difficult. To determine the time needed to create a PTSL test site was investigated. In 1994, a site not planted with peaches for >80 years was identified in Byron, GA. Preplant nematode soil samples revealed no Mx. One third of the land was planted to peach and infested with Mx in spring 1994 (P2) and another third in spring 1995 (P1). The remaining third of the land received no trees or Mx and served as the untreated control. In winter 1995, trees were removed from P1 and P2

and all treatments were replanted to peach in 1996. In 1997, PTSL tree death only occurred in P2 (7%). By 2000, PTSL tree death reached 41% in P2, 16% in P1, and 4% in the control. Nematode populations were greatest ($P < 0.05$) in P1 (649 Mx/100 cc soil) and P2 (300 Mx/100 cc soil) and lowest in the control (221 Mx/100 cc soil). Establishing a PTSL screening site is possible after three years.

EVALUATION OF BAERMANN FUNNEL EXTRACTION FOR NEMATODE COMMUNITY STUDY. H. OKADA.
Tohoku National Agricultural Experiment Station.

To examine availability of Baermann funnel extraction for nematode community study, nematodes were extracted by Baermann funnel (BF) and sieving+centrifuge (SC) from soils of three sites: tilled (T) and non tilled (N) crop fields, and a forest (F). Density of each nematode taxon was estimated to calculate diversity (λ and H') and ecological (MI and PPI) indices in each sample, and the indices were compared with regard to extraction method and study site. The nematode numbers were significantly larger in samples extracted by BF than by SC. The nematode density was significantly higher in F than in T or N. The study sites ranked for community indices as follows: F>T>N in both BF and SC extractions for MI; T>N>F in BF but T>F>N in SC for PPI; F>T>N in both BF and SC for (I); N>T>F in BF but N>F>T in SC for H' . In conclusion, BF may be a useful method same as SC in revealing differences in nematode communities at least between T and N.

A FUNGAL-FEEDING NEMATODE IN THE FAMILY TYLENCHIDAE FROM DECOMPOSING RICE STRAW. H. OKADA.
Tohoku National Agricultural Experiment Station.

In autumn 2000, a stylet-bearing nematode species was collected from decomposing rice stems and leaves in Fukushima City, Japan. The nematode had general characteristics of the Tylenchidae, with the following morphometrics: females ($n = 22$); $L = 357.2 \mu\text{m}$, $a = 27.5$, $b = 4.5$, $c = 5.7$, $c' = 7.9$, $V = 69.2$, stylet = $7.2 \mu\text{m}$, MB = 47.6, oesophagus = $79.8 \mu\text{m}$, E. pore = $61.2 \mu\text{m}$, tail = $63.2 \mu\text{m}$, deirid = $67.7 \mu\text{m}$, spermathecae were round and offset, containing sperm with little cytoplasm; males ($n = 5$); $L = 330.4 \mu\text{m}$, $a = 34.9$, $b = 4.1$, $c = 5.3$, $c' = 7.9$, T = $39.2 \mu\text{m}$, stylet = $6.8 \mu\text{m}$, MB = 48.5, oesophagus = $80.8 \mu\text{m}$, E. pore = $60 \mu\text{m}$, tail = $61.8 \mu\text{m}$, deirid = $64.0 \mu\text{m}$, spicule = $13.2 \mu\text{m}$, gubernaculum = $3.8 \mu\text{m}$. Based on these characteristics the nematode was identified as *Filenchus* sp. (Tylenchidae). On water agar and PDA, the nematode fed on hyphae of fungi isolated from the decomposing rice straw, and laid eggs. Determination of the fungal-feeding habit of this nematode may be important for studies on the functional ecology of soil nematodes and in their use as bioindicators of environmental condition.

GENETIC ANALYSIS OF SOYBEAN CYST NEMATODE PARASITISM. C. OPPERMAN. North Carolina State University, Raleigh, NC 27604.

During the past several years, we have developed a forward genetic system using the soybean cyst nematode (*Heterodera glycines* - SCN) as a model parasite. Forward genetic analysis has revealed that the parasite contains multiple unlinked loci that enable it to evade or circumvent host resistance genes, termed *ror* genes for "Reproduction On a Resistant" host. These individual *ror* genes appear in either dominant or recessive states. Controlled crosses have been performed between several SCN strains. Segregation analyses have revealed that inheritance of parasitic ability is Mendelian in nature. The genes controlling parasitic ability on a specific host differential appear to be unlinked loci. The SCN gene controlling ability to parasitize PI 88788 is inherited as a dominant allele, whereas the genes conferring parasitic ability on PI 90763 and Pickett are recessive. Genetic mapping of these loci places them at the ends of separate linkage groups, known to be recombinationally active areas in the free-living nematode *Caenorhabditis elegans*. Analysis of one of these areas in *H. glycines* reveals the presence of mobile genetic elements closely associated with the *Ror-1* locus. A genetic map of the *H. glycines* genome has been constructed. The 92.5 Mb genome has nine linkage groups. Construction of a physical map by BAC fingerprinting is under way and will be complete soon. In addition to the *ror* genes, we have placed ESTs on the map, including those with homology to *C. elegans*.

INTEGRATION OF GIS AND REMOTE SENSING FOR PERENNIAL CROP MANAGEMENT. P. V. OUDEMANS.
Dept. of Plant Pathology, Rutgers University, 125a Lake Oswego Rd., Chatsworth, NJ 08019.

Woody perennial crops, such as cranberries and blueberries exhibit tremendous variability in crop yields and quality as imposed by variations in soil properties and crop stress (diseases and weed competition). These factors result in persistent yield losses and crop quality reductions. To identify losses and develop crop management strategies, state of the art methodologies (GIS, GPS, remote sensing) are used to quantify and map spatial variation of crop yield. Hyperspectral aerial remote sensing is inter-related with ground-sampled data using geostatistical methods to identify stress and quantify yield variation. Ultimately, development of accurate yield maps will provide field professionals with sufficient information to classify crop loss and take action during a growing season. The information is delivered as user-friendly maps accessible in a digital format and can be downloaded to hand-held computers for easy in-field reference. Issues currently being tackled

include the timing and frequency of aerial over flights as well as the spatial and spectral resolution necessary for accurate interpretation of crop condition.

TEM OBSERVATIONS OF ROOT-KNOT NEMATODE RECTAL GLAND CELLS AND RELATED TISSUES. D. Orion (1), W. P. WERGIN (2), C. A. Murphy (2), and D. J. Chitwood (2). (1) Dept. of Nematology, ARO, Volcani Center, Bet-Dagan 50250, Israel; (2) USDA, ARS, Beltsville, MD.

Transmission electron microscopy of the posterior regions of female *Meloidogyne incognita* revealed features of the rectal gland cells (RGCs), the gelatinous matrix (GM), and other tissues. Each RGC, typically >50 μm long, contains a prominent nucleus and nucleolus. Rough ER and glycogen granules are the most common organelles in the anterior region of RGCs, whereas the posterior contains an elaborate system of ducts and vesicles. The vesicles contain granular material similar in appearance to that of the GM and secrete their contents into the ducts leading to the anus. The granular material progresses to the exterior of the female and engulfs the developing eggs. The hypodermal cells near the rectal glands are elongated and contain prominent nuclei, dense cytoplasm and many glycogen granules. The adjacent somatic muscles are degenerated. The vulva is a valve with a thick cuticle and massive muscles that connect to the cuticle and contract to open the vulva. These structural features assist in visualizing the processes leading to GM development and egg laying.

TELONE II FOR THE MANAGEMENT OF THE RENIFORM NEMATODE IN COTTON DURING 1999-2000 IN NORTHERN LOUISIANA AND SOUTHERN MISSISSIPPI. C. OVERSTREET (1), E. C. McGawley (2), and G. W. Lawrence (3). (1) LSU Agricultural Center, LA Coop. Ext. Serv.; (2) Dept. Plant Pathology and Crop Physiology, LSU; (3) Dept. Ent. and Plant Pathology, Miss. State Univ.

Reniform nematode has become a difficult pest to manage in many cotton fields because of continuous cotton production and limited rotational options. To evaluate Telone II as a management option, three large-field nematicide trials were conducted in fields heavily infested by reniform nematode in northern Louisiana and one in southern Mississippi. Cotton yield significantly increased with the addition of Telone II when compared with the grower standard of aldicarb. Yield increases of cotton ranged from 4 to 37 percent when Telone II at 34 kg a.i./ha was added to the standard rate of aldicarb (0.59 kg a.i./ha). Telone II significantly reduced mid-season populations of reniform nematode compared to the standard only at one location and at none of the locations with the final nematode levels. The application of Telone II may offer significant yield increases and serve as an important management practice against high levels of reniform nematode.

EFFECT OF ENTOMOPATHOGENIC NEMATODES ON ROOT PENETRATION AND EGG PRODUCTION OF MELOIDOGYNE INCOGNITA ON TOMATO SEEDLINGS. E. E. PEREZ and E. E. Lewis. Entomology Department, VPI and SU, Blacksburg, VA 24061.

The effect of *Steinernema feltiae*, *S. riobrave*, *S. carpocapsae*, *Heterorhabditis bacteriophora*, and *H. megidis* infective juveniles (IJ) on *Meloidogyne incognita* egg production and root penetration on tomato seedlings was tested. Each entomopathogenic nematode species was applied at a rate of 25 IJ/cm² together with 150 eggs of *M. incognita* to tomato seedlings (approximately 10 cm tall) grown in sterilized sand. Two weeks after treatment, control seedlings had more *M. incognita* inside the roots than seedlings treated with *S. riobrave* ($p < 0.05$), *S. feltiae* ($p < 0.10$), and *H. megidis* ($p < 0.10$). Five weeks after treatment, the number of eggs extracted from control seedlings was higher than those extracted from seedlings treated with *S. riobrave* ($p < 0.05$), *S. feltiae* ($p < 0.05$), *H. bacteriophora* ($p < 0.10$), and *H. megidis* ($p < 0.10$).

TIMING APPLICATION OF STEINERNEMA FELTIAE ON TOMATO PLANTS TO SUPPRESS MELOIDOGYNE INCOGNITA. E. E. PEREZ and E. E. Lewis. Entomology Department, VPI and SU, Blacksburg, VA 24061.

Rates of 25 or 125 *Steinernema feltiae* /cm² were applied at weekly intervals from two weeks before tomato plants were infested with 4,000 *Meloidogyne incognita* eggs to two weeks after *M. incognita* infestation. Roots were stained two weeks after *M. incognita* infestation to assess nematode penetration. Control plants had more *M. incognita* than plants treated with 25 *S. feltiae*/cm² one week before ($p < 0.05$), simultaneously ($p < 0.05$), and two weeks before ($p < 0.10$) *M. incognita* egg infestation. Five weeks after *M. incognita* infestation control plants had more *M. incognita* eggs than plants treated with 25 *S. feltiae*/cm² two weeks before ($p < 0.05$), simultaneously ($p < 0.05$), one week before ($p < 0.10$), and one week after ($p < 0.10$) *M. incognita* infestation. The rate of 125 *S. feltiae*/cm² had no effect on *M. incognita* root penetration or egg production.

FREQUENCY OF VIRULENCE TO COWPEA GENE Rk IN ISOFEMALE LINES OF MELOIDOGYNE INCOGNITA. M. D. PETRILLO and P. A. Roberts. Dept. Nematology, University of California, Riverside.

The root-knot nematode, *Meloidogyne incognita*, reproduces by obligate mitotic parthenogenesis and is polyploid. The *Rk* gene in cowpea, *Vigna unguiculata*, confers resistance to *M. incognita* though virulent isolates to gene *Rk* have been reported. Twelve isofemale lines (IFLs) of *M. incognita*, each being an isolate derived from a single female, were evaluated

for virulence to *Rk* over 23 generations. Five distinct profiles for (a)virulence were observed. Four profiles were represented by a stable avirulent condition, a stable virulent condition, selection from the avirulent to the virulent condition when selected on *Rk*, and decline in virulence from the virulent to the avirulent condition when continuously cultured in the absence of *Rk* on susceptible plants. A fifth profile represented changes in virulence to *Rk* when continuously cultured in the absence of *Rk*. Some IFLs became extinct when attempting to select for virulence to *Rk* and other IFLs became extinct when continuously cultured in the absence of *Rk* on susceptible plants. The influence of host selection on the occurrence of the virulent phenotype in *M. incognita* is discussed.

EVOLUTION OF INSECT PARASITISM IN MERMITHIDS. E. G. PLATZER. Department of Nematology, University of California, Riverside, CA 92521.

Analysis of the evolutionary history of invertebrate groups is dependent on preservation of past life and activities. As a consequence of the relatively delicate nature of nematodes, there is a void in the classic lithic fossil records. However, fossiliferous amber has provided a record for mermithid nematodes from the Mesozoic and Cenozoic. Further biogeographic-paleogeologic studies by Poinar have extended the potential evolutionary record for mermithid life history patterns to the late Paleozoic. In addition to the fossil record for early development of mermithid parasitism, there is evidence provided by the marimermithids for early development of the life history characteristics of parasitism as a juvenile in invertebrates for enoplids. Development of an ephemeral odontostyl within the mermithids may also reflect the potential of microbivory in the development of invertebrate parasitism. Further studies on the developmental history and the evolutionary relationships of specific gene products within the enoplids should facilitate the understanding of the evolution of life history characteristics of mermithid nema.

TERMINATION OF OBLIGATE DEVELOPMENTAL DORMANCY IN AN ASCARID. E. G. PLATZER (1), L. T. Luong (2), and N. Hinkle (3). (1) Dept. of Nematology, University of California, Riverside, CA 92521; (2) Dept. of Nematology, University of California, Davis, CA 95616; (3) Dept. of Entomology, University of California, Riverside, CA 92521.

Allodapa suctoria is a subulurid nematode in the Order Ascaridida. As an adult it is common in the cecum of poultry. Infective juveniles (J2) are encapsulated in the hemocoel of coleopterans, dermapterans, and orthopterans. Encapsulated J2's were obtained by dissection from *Alphitobius diaperinus* (darkling beetle) and placed in physiological saline at 38 C in a 95 percent nitrogen: 5 percent carbon dioxide atmosphere. No excystment took place. In a second experimental series, the J2's were released from the capsule when trypsin was included in the saline. However, the J2's remained inactive. Inclusion of bile salts (sodium tauroglycolate) in the incubation conditions used in the second experiment resulted in the release of active J2's. In this case, bile salts were the physiological trigger required to terminate the obligate developmental dormancy in the J2's of *Allodapa suctoria*.

BIOFUMIGATION AND SOIL HEATING TO CONTROL MELOIDOGYNE INCOGNITA AND M. JAVANICA. A. T. PLOEG. Department of Nematology, University of California, Riverside, CA 92521.

Plastic vials (250 ml) were filled with *M. incognita* or *M. javanica* infested sand. Treatments consisted of adding or not adding finely chopped fresh Broccoli leaf and stem material (2 g/100 g sand) to the sand, and placing the vials in waterbaths at 40, 35, 30, 25 and 20 C. Vials were removed from the waterbaths after 1, 3, 10, 15 and 20 days, melon (var. Durango) was then seeded in each vial and the melon seedling was grown for 4 weeks. After 4 weeks, root and top fresh weight, root galling, and number of egg masses were recorded. Results were similar for *M. incognita* and *M. javanica*. At temperatures above 25 C, addition of broccoli plant refuse to the soil significantly reduced galling of melons. In a related study on a *M. incognita* infested field, the combination of solarization and incorporation of broccoli refuse, reduced melon root galling and population levels at harvest, and increased fruit yield compared to the non-treated fallow control.

HOST STATUS AND SUSCEPTIBILITY OF LISIANTHUS TO THREE MELOIDOGYNE SPECIES. A. T. PLOEG. Department of Nematology, University of California, Riverside, CA 92521.

Lisianthus (*Eustoma grandiflorum*), a plant native to the USA, is gaining in popularity in the floriculture industry. Root-knot nematodes were suspected as a cause of poor stand, yellowing and wilting of Lisianthus in greenhouse operations in Southern California. As information on the host status and susceptibility of Lisianthus to root-knot nematodes is not available, greenhouse experiments were initiated to provide such information. Transplants of Lisianthus var. Mariachi - Lime Green or four-week-old tomato var. Tropic seedlings were planted into pots containing 3 kg steam-sterilized soil. One week later, second-stage juveniles (J2) of the root-knot nematodes *Meloidogyne incognita*, *M. javanica* or, *M. hapla* were added to the pots (10^2 , 10^3 , 10^4 juveniles per pot). Pots without nematodes were included as controls. Data on the host status and susceptibility of Lisianthus to the three *Meloidogyne* species will be presented and compared to tomato.

FUNCTIONAL ANALYSIS OF A NEMATODE INDUCED CELL CYCLE GENE, CDC2A, THROUGH ANTISENSE AND COSUPPRESSION. **E. PLOVIE (1), E. Huyck (1), and G. Gheysen (1,2).** (1) VIB, Dept of Plant Genetics, Ghent university, K.L Ledeganckstraat 35, 9000 Ghent, Belgium; (2) Present address: Faculty of Agricultural and Applied Biological Sciences, Ghent University, Coupure links 653, 9000 Ghent, Belgium.

Sedentary plant parasitic nematodes are economically important because they cause yield and quality losses in many crops. Juveniles emerging from the eggs infect plant roots by penetration, migration to specific tissues and they induce a redifferentiation of plant root cells into specialised feeding cells. These feeding cells are indispensable for the growth and reproduction of the nematodes. Cyst nematodes generate syncytia by cell wall degradation and root-knot nematodes induce giant cells by mitosis without cytokinesis. Feeding site formation by cyst and root knot nematodes is accompanied by cell cycle activation and the multinucleate nature of these feeding cells is generated by incomplete cell cycles. During the last years several cell cycle genes have been identified that show much higher expression level in the nematode feeding cells compared to normal root cells. In this study we want to inhibit the expression of a cell cycle gene, *cdc2aAt*, that is strongly activated in the feeding sites, by antisense and cosuppression. In this way we hope to block the formation or the functioning of the feeding cells, so we can learn something about the role of the cell cycle and in the mean time try to make the plant resistant against infection by sedentary nematodes. Several constructs have been made and transformed in *A. thaliana*. Nematode resistance tests with the cyst nematode *Heterodera schachtii* and the root-knot nematode *Meloidogyne incognita* were performed and the most interesting lines will be analyzed at the molecular level.

TRENDS IN THE EVOLUTION OF INSECT PARASITISM BY NEMATODES. **G. O. POINAR, Jr.** Department of Entomology, Oregon State University, Corvallis, OR 97331.

The duration of insect parasitism by specific nematode groups can be established with fossils. Discoveries in amber show parasitism by Mermithidae in the Early Cretaceous, by Tetradonematidae, Sphaerulariidae and rhabditids in the Eocene and by Allantonematidae and Diplogasteridae in the mid-Tertiary. While there are no confirmed fossils of entomopathogenic nematodes, it is likely that their association with arthropods extended back to the Paleozoic. The evolutionary history of entomopathogenic nematodes is complicated since it involves the selection of a bacterium that can be utilized as a food source, the retention of that bacterium by the dauer and the ability to invade the arthropods body cavity. With the Heterorhabditidae, which appears to have evolved from a *Pellioidites*-like ancestor in an arenicolous marine habitat, parasitism initially may have involved littoral crustaceans as well as intertidal insects. Evolution of the Steinernematidae appears to have occurred in a completely terrestrial environment from an early member of the *Rhabditophanes* clade.

EVALUATING THE POTENTIAL FOR ANTAGONISM BETWEEN AN APHID PREDATOR AND ENTOMOPATHOGENIC NEMATODES. **J. R. POWELL and J. M. Webster.** Dept. Biological Sciences, Simon Fraser Univ.

Antagonism (an interaction between members of different species that reduces the ability of one or both agents to manage a host population) can occur in integrated pest management systems if the control agents are not compatible. An inundative application of entomopathogenic nematodes (EPNs) generally is believed to have no impact on beneficial arthropod populations, but this assumption has not been rigorously tested. The aphid midge, *Aphidoletes aphidimyza*, is used to manage aphids in greenhouse, orchard, and field crops, and several stages in its life cycle are vulnerable to EPN infection. Filter paper bioassays indicate that the level of aphid midge mortality differs with EPN species applied (>80% for *Steinernema carpocapsae*, <25% for *Heterorhabditis bacteriophora*; ~500 IJs for 5 days), and is lower if the midge is cocooned during exposure (>20% (uncocooned), <2% (cocooned) for *Steinernema feltiae*; ~500 IJs). Non-target insects, such as the aphid midge, may act as alternate hosts and/or disrupt host cue gradients, thus interfering with target-host finding by EPNs. The potential for antagonism in the EPN-aphid midge system will be discussed.

EVOLUTION OF PLANT PARASITISM IN NEMATODES. **T. O. POWERS (1), B. J. Adams (2), P. G. Mullin (1), and A. L. Szalanski (1).** (1) Dept. Plant Pathology, Univ. Nebraska-Lincoln, 406 Plant Science, Lincoln, NE 68583; (2) Dept. of Entomology and Nematology, Univ. of Florida, Gainesville, FL 32611.

It is generally agreed that plant parasitism evolved independently on several occasions during the course of nematode evolution. The nature of plant-parasitic nematode ancestors has been a fertile topic of speculation among systematists. Proposed progenitors of plant-parasites include algal and fungal feeders, as well as predaceous nematodes. Some of these taxa include *Hexatyclus*, *Psilenchus*, anguinid, aphelenchid, cephalobid, and diplogasterid nematodes. Phylogenetic trees based on molecular characters provide a means to independently test different contrasting theories regarding the origin of plant parasitism. The expanding 18S ribosomal DNA tree serves as a framework to examine the relationship of nematode taxa believed to be involved in early evolutionary stages of plant parasitism.

PASTEURIA SPP.: SYSTEMATICS-PHYLOGENY OF THESE UNUSUAL BACTERIAL PARASITES OF PHYTOPATHOGENIC NEMATODES. **J. F. PRESTON (1), D. W. Dickson (2), J. E. Maruniak (2), J. A. Brito (2), L. M. Schmidt (1), and J. M. Anderson (2).** (1) Microbiology and Cell Science Dept.; (2) Entomology and Nematology Dept., University of Florida, Gainesville, FL 32611.

Pasteuria spp. 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. Efforts in three laboratories have sequenced the 16S ribosomal RNA genes from *Pasteuria* isolated from root-knot (*Meloidogyne* spp.), soybean cyst (*Heterodera glycines*), and sting (*Belonolaimus longicaudatus*) nematodes, allowing their designation as distinct but closely related species within a clade of endospore-forming bacteria, including the cladoceran parasite, *P. ramosa*. Isolates of *P. penetrans* from different *Meloidogyne* spp. showed identical sequences, indicating host-recognition evolves within a given species. Distribution of an adhesin epitope on polypeptides from *Pasteuria* isolates provides an immunochemical approach to differentiate isolates with specific host preferences for biocontrol applications.

INTERACTION BETWEEN THE RENIFORM NEMATODE AND THRIPS ON MISSISSIPPI COTTON. W. O. PRICE, G. W. Lawrence, and J. T. Reed. Department of Entomology and Plant Pathology, Mississippi State University, Mississippi State, MS 39762.

Tests were established in field micro plots to examine the interaction between the reniform nematode (*Rotylenchulus reniformis*) and thrips on seedling cotton. Cotton cv. Bollgard 33B was inoculated with initial population numbers (Pi) of 0, 500, 1000 and 5000 reniform nematodes / 500 cm³ of soil. When plants emerged, thrips were infested at Pi = 0, 2, 5 and 10 thrips / plant and immediately caged. At harvest, plant height, total nodes, location of bolls by node and position, number of aborted terminals, number of vegetative bolls and cotton yields were recorded. Initial population levels of the reniform nematode and thrips had a significant effect on cotton plant growth parameters. Both the reniform nematode and thrips reduced the number of nodes per plant. A greater number of cotton plants with aborted terminals were recorded in the reniform infested plots. Seed cotton yields decreased as the reniform Pi levels increased. No significant interaction between the reniform nematodes and thrips was observed for seed cotton yields.

REDUCTION OF ROOT-KNOT NEMATODE, *M. JAVANICA*, IN SOIL TREATED WITH OZONE. J. J. QIU (1), B. B. Westerdahl (1), A. Pryor (2), and C. E. Anderson (1). (1) Dept. Nematology, University of California, Davis, CA 95616; (2) SoilZone, Inc., Davis, CA 95616.

Ozone gas (O₃), has been extensively used to disinfect water. O₃ rapidly breaks down into oxygen following application. The use of O₃ for soil-fumigation was investigated in laboratory trials. O₃ was produced at a concentration of 1% in air by a conventional electrical discharge O₃ generator. In preliminary trials, O₃ mass transfer rate was influenced by soil texture and moisture levels. Two ozone dosage levels and 3 gas flow rates were tested against resident populations of *M. javanica* (MJ) and free-living nematodes (FL) in a field collected sandy loam soil with a moisture level of 11% (w/w). FL consisted of 58% Cephalobidae, 21% Diplogasteridae and 5 additional families. Survival was assessed with Baermann funnel. Results from two replicated trials were consistent. The reduction for both MJ and FL was dosage dependent and flow rate independent. Compared with untreated, at a dosage equivalent to 250 kg/ha, MJ and FL, were reduced by 68% and 52% ($P = 0.05$), respectively. At a dosage equivalent to 50 kg/ha, reductions were 24% and 19% ($P = 0.05$), respectively.

DEVELOPMENT OF AN ORGANIC PESTICIDE BASED ON NEEM TREE PRODUCTS. E. RIGA and G. Lazarovits. Agriculture & Agri-Food Canada.

The purpose of this project is to develop information on the biological activity of Neem products against a wide spectrum of North American plant pathogenic fungi and bacteria; plant parasitic nematodes; and beneficial free-living nematodes. Toxicity tests in Petri dish bioassays in which plant parasitic nematode juveniles were treated with 1% Neem Cake water extract showed that their survival rate was significantly lower than the survival rate of the untreated controls. Similarly, the survival rate of free-living nematode juveniles was significantly lower than their controls. Similar nematode survival rates were obtained from bioassays in which 1% Neem Cake was added to soil. Neem Cake caused higher mortality to plant parasitic nematodes in comparison to the beneficial free-living nematodes, thus making it a desirable organic amendment as it does not have detrimental effect on soil free-living beneficial organisms. Azadirachtin, the biologically active component of Neem, repels all of the above nematodes in behavioural bioassays, and inhibits fungal and bacterial growth. In addition, soil analysis showed that Neem is converted to ammonia in the soil, 10 days post-application. Neem appears to have a multiple mode of action against plant pathogenic organisms by causing repellency, inhibition and mortality.

EFFECT OF CONTAINER ON SOYBEAN CYST NEMATODE RACE TESTS AND INDICES ON THE DIFFERENTIALS. R. D. RIGGS and L. Rakes. Dept. Plant Pathology, Univ. of Arkansas.

Race tests were conducted with races 2 and 9 of soybean cyst nematode, *Heterodera glycines*, in three different containers, clay pots 7.5 cm diam. by 8.2 cm deep, styrofoam cups 6.7 cm diam. by 7.7 cm deep and cone-tainers 2.5 cm diam. by 16.0 cm deep. Seedlings were transplanted into fine sandy loam soil and 4,000 eggs + 2nd-stage juveniles were added to each container. After 30 days, soil and roots in each container were processed to extract mature females, which

were counted with a stereomicroscope. Number of race 2 females produced was significantly higher in pots (753) than in cups, (549) or cone-tainers (526). Cultivars Lee and Pickett had higher numbers of females, regardless of container, than other differentials. In pots, female numbers were not different on Lee and Pickett but were different in cups and cone-tainers. Race 9 produced more females in cone-tainers (240) than in pots (177) or cups (175). Lee and Pickett did not always have more females than Peking but had more than the other differentials in all containers. In cups, female numbers were different with Lee Pickett Peking PI 88788 and PI 90763. The relationships were not the same in the other two containers.

RENIFORM NEMATODE REPRODUCTION ON SOYBEAN IN TESTS CONDUCTED IN 2000. R. T. ROBBINS, L. Rake, L. E. Jackson (1), E. E. Gbur (2), and D. G. Dombek (3). (1) Plant Pathology Dept.; (2) Agricultural Statistics Laboratory; (3) Arkansas Crop Improvement Program, University of Arkansas.

Reproduction of reniform nematode (*Rotylenchulus reniformis*) on 118 soybean lines were greenhouse tested, 115 were new in the Arkansas and Mississippi Soybean Testing Programs and 3 were from Texas. Controls were resistant lines Forrest and Hartwig, susceptible Braxton, and fallow infested soil. Five treatment replications were planted in sandy loam soil infested with 2,256 eggs and vermiform reniform nematodes, grown for 11 weeks in 10 cm-dia pots. Total reniform nematodes extracted from soil and roots was determined and a reproductive factor (final population (Pf)/initial inoculum level (Pi)) was calculated for each line. Also, reproduction on each line was compared to the reproduction on the resistant cultivar Forrest (Pf cultivar/Pf Forrest). Analysis of variance was conducted using the log₁₀ of these ratios. Delta Grow 5940 and Padre, and the resistant control Hartwig were not different (P 0.05) from Forrest in final nematode numbers. Missouri lines S94-1867, S96-2692, and S96-2641, descended from Hartwig, were comparable to Forrest.

DIFFERENCES IN COTTON YIELD, ROOT GROWTH, AND ROTYLENCHULUS RENIFORMIS FOLLOWING DEEP SOIL FUMIGATION. A. F. ROBINSON (1), C. G. Cook (2), J. M. Bradford (1), A. C. Bridges (1), and J. Bautista (1). (1) USDA-ARS; (2) Syngenta, Inc.

Recent cotton surveys have shown highest population densities of *Rotylenchulus reniformis* frequently more than 50 cm deep. To investigate the impact and origin of deep *R. reniformis*, soil in a field at Weslaco, TX, was fumigated in November by digging post holes 90 cm deep and placing 2 ml of 1,3-dichloropropene 90, 60 and 30 cm deep as holes were refilled. Altogether, 14 fumigated holes were spaced on 51-cm centers along the bed and the adjacent furrow. Fumigant-free post holes, unfumigated chiseled areas, and chisel-fumigated areas were included. In the spring, fumigation to 90 cm had killed most *R. reniformis* down to 105 cm and 50-100 cm laterally whereas chisel fumigation 43 cm deep killed nematodes only in the top 60 cm. At cotton harvest in July, root density was always highest near the surface and more or less uniform from 30 to 105 cm. The most striking effect was continued nematode population control at harvest at all depths following deep fumigation, compared with complete resurgence of nematode populations in chisel-fumigated plots. Fumigation doubled yields in both cases.

PHYLOGENETIC RELATIONSHIPS OF AN UNDESCRIBED SPECIES OF GLOBODERA FROM PORTUGAL AND TWO PUNCTODERA SPECIES BASED ON RIBOSOMAL DNA SEQUENCE DATA. A. SABO (1), L. G. L. Reis (2), E. Krall (3), M. Mundo-Ocampo (4), and V. R. Ferris (1). (1) Dept. Entomology, Purdue Univ., W. Lafayette, IN 47907; (2) Dept. Fitopatologia, Estação Agronómica Nacional, 2784-505 Oeiras, Portugal; (3) Inst. Zoology and Botany, Riia 181, 51014 Tartu, Estonia; (4) Dept. Nematology, Univ. California, Riverside, CA 92521.

Evolutionary relationships based on ribosomal DNA (rDNA) data for an undescribed species of *Globodera* from Portugal that parasitizes the Compositae, *Chamaemelum mixtum*, *Punctodera chalcoensis* from Mexico, and *P. punctata* from Estonia, along with published rDNA data for other nematode species, support the following relationships: (((*C. weissi*, *G. artemisiae*), *C. milleri*), ((*G. sp.* Bouro, *G. sp.* Canha, *G. sp.* Ladoeiro), ((*G. pallida*, *G. rostochiensis*), (*P. chalcoensis*, *P. punctata*))), *H. avenae*). The undescribed *Globodera* species from three localities in Portugal can be distinguished from the potato cyst nematodes, and is only distantly related to other European *Globodera* species that parasitize Compositae. *Punctodera chalcoensis* and *P. punctata* form a sister clade to the *Globodera pallida* + *G. rostochiensis* group, based on their rDNA.

USING TORUG TO DISSECT HOST RESPONSES TO KNOT INFECTION. J. SCHAFF, E. Scholl, and D. Bird. PNGG, NCSU, Raleigh, NC.

We are interested in global changes in host gene expression following root-nematode infection, and previously used a subtractive, cDNA cloning approach to identify approximately 200 tomato genes differentially expressed at nematode feeding sites. We routinely scour the databases for their homologues, and are experimentally analyzing genes with interesting identities. To find additional candidates we have initiated a microarray screen. We identified a set of EST clones defining approximately 4,300 unique genes expressed in tomato roots in the TiGR database. Clustered ESTs were searched

to identify the longest sequences for each gene, and these clones were re-arrayed into the Tomato Root Unigene (ToRuG) set. The ToRuG library is a dynamic assembly, with new sequences being added as they are discovered. ToRuG clones will be merged with clones from the subtracted cDNA library, along with clones encoding genes of known and interesting identities, and this set will be used to construct micro-arrays. Using these arrays we hope to define temporal changes in tomato root gene expression associated with root-knot infection, and also to identify differences in gene expression induced by various species of *Meloidogyne*.

SURVEY TO DETERMINE NEMATODES ASSOCIATED WITH COFFEE IN HAWAII. D. P. SCHMITT (1), M. Serracin (1), and N. V. Hue (2). (1) Department of Plant and Environmental Sciences; (2) Department of Tropical Plant and Soil Sciences, University of Hawaii, Honolulu, HI 96822.

A survey of nematodes on coffee was conducted from 1999-2000 in Hawaii on the islands of Kauai, Oahu, Molokai, Maui, and Hawaii. Sampling sites were selected using a randomized stratified sampling design. *Paratylenchus* sp. was the dominant plant-parasitic nematode on the 2,000 hectares of coffee on Kauai. On the 70 hectares of coffee on Oahu, the most common nematodes were a putative new genus similar to *Fergusobia* and a putative new species of *Pratylenchus*. This same lesion nematode occurred in all samples from the 200 hectares of coffee on Molokai. A putative new species of *Meloidogyne* occurred in 65% of the 300 hectares of coffee on Maui. On the island of Hawaii, 85% of the 1,000 hectares of coffee are infested with *Meloidogyne konaensis*. Germplasm plots located on Oahu were infested with *Meloidogyne incognita*. Economic loss due to some of these species is high. The losses are exacerbated by nutritional deficiencies or excesses and by some cultural practices.

FLUORESCENCE CORRELATION SPECTROSCOPY FOR IN VIVO MONITORING OF RESISTANCE MECHANISMS. A. SCHOTS (1), R. Dees (1), A. Goverse (2), J. W. Borst (1,3), J. Bakker (2), and A. J. W. G. Visser (3). (1) Lab. of Molecular Recognition and Antibody Technology; (2) Lab. of Nematology; (3) Microspectroscopy Centre, Wageningen University, Wageningen, The Netherlands.

Using fluorescence correlation spectroscopy (FCS) molecular interactions at the single-molecule level can be investigated. Molecular binding events and molecular processing can be measured. Important techniques in this respects are fluorescence resonance energy transfer (FRET) measured by fluorescence lifetime imaging microscopy (FLIM) and two colour fluorescence cross-correlation spectroscopy (FCCS). Using multiphoton lasers these techniques are now also applicable for measurements in plant cells. To gain insight in protein interactions usually protein fusions with yellow (YFP) and cyan (CFP) variants of the green fluorescent protein are used. These technologies are applied to gain insight in the resistance mechanism of the homologous resistance genes *Rx* and conferring resistance to potato virus X and *Globodera pallida*.

GROWTH AND YIELD OF COFFEE AS INFLUENCED BY IRRIGATION, TREE AGE AND MELOIDOGYNE KONAENSIS. M. SERRACIN (1), D. P. Schmitt (1), and B. S. Sipes (1). (1) Department of Plant and Environmental Protection Sciences, University of Hawaii at Manoa, Honolulu, HI 96822.

Meloidogyne konaensis (MK) causes coffee decline in Hawaii. Irrigation and age of seedling transplant may alter the damage. The effect of these two cultural practices on coffee growth and yield was evaluated at a research farm in Kainaliu, Kona, Hawaii over two years. Treatments were arranged in a 2x2x2 factorial. Treatments were: *Coffea arabica* cv. 'Typica' transplant age (6-month and 12-month old seedlings), irrigation (+,-) and nematodes (+,-). The pattern of plant growth was similar among treatments, but rate of growth varied. The slowest growth rate occurred with 6-month old transplants infected with MK and irrigated. One-year-old seedlings which were irrigated and free of MK grew the most vigorously. In 1999, irrigation had the greatest effect on yield in comparison to other treatments. In 2000, the highest yield occurred in nematode free irrigated plots planted with 1-year-old seedlings. MK suppressed yields by 31% to 43%. MK damage to coffee roots is influenced by the complex interaction of root abundance and amount of soil moisture.

VIRULENCE OF ENTOMOPATHOGENIC NEMATODES TO THE PECAN WEEVIL, CURCULIO CARYAE, IN THE LABORATORY. D. I. SHAPIRO-ILAN. USDA-ARS, SE Fruit and Tree Nut Research Lab, Byron, GA 31008.

The pecan weevil, *Curculio caryae*, is a key pest of pecans in the Southeast. Prior to this research, adult weevils had not been evaluated for susceptibility to entomopathogenic nematodes and only three species of nematodes had been tested against pecan weevil larvae. In this study, we tested the virulence of 9 species and 15 strains of nematodes towards fourth instar pecan weevil and 4 species (5 strains) were tested against adult weevils. Nematode virulence toward pecan weevil larvae was low-moderate and did not differ significantly among nematode strains. Results indicate that as pecan weevil larvae age they may have become more resistant to infection with entomopathogenic nematodes. Pecan weevil adults were found to be more susceptible to entomopathogenic nematodes than weevil larvae. *Steinernema carpocapsae* showed particular promise and caused close to 100% adult weevil mortality after four days of exposure.

ANALYSIS OF ELECTRICAL ACTIVITY IN POTATO ROOTS IN RESPONSE TO THE POTATO CYST NEMATODE *GLOBODERA ROSTOCHIENSIS*. **J. P. SHERIDAN, A. J. Miller, and R. N. Perry.** IACR-Rothamsted, Harpenden, Hertfordshire, AL5 2JQ, UK.

Electrical signals have been implicated in early plant responses to attack by various plant pathogens, but this phenomenon has not been tested during nematode invasion. We have developed a novel technique for comparing root cell electrical activity in resistant and susceptible potato cultivars before, during and after invasion by *G. rostochiensis*. The stable resting membrane potentials of epidermal cells, 0.1 to 0.5 cm from the root tip, of Desiree (susceptible) and Maris Piper (resistant) were significantly different at -120 ± 5 mV and -100 ± 4 mV, respectively. After applying nematodes, electrical spike activity was recorded from root cells. The pattern of electrical activity was the same from roots of both cultivars. The numbers of nematodes invading were similar in both cultivars, but after 3 days no feeding sites had been established in Maris Piper, whereas 50% of the invading juveniles had successfully formed feeding sites in Desiree. Thus, both the resting membrane potential and the electrical activity during the invasion process appear not to have a role in plant resistance to nematode attack.

AN OVERVIEW OF EVOLUTION OF PARASITISM IN NEMATODES. **M. R. SIDDIQI.** Department of Entomology & Nematology, IACR-Rothamsted, Harpenden, Herts., AL5 2JQ, United Kingdom.

Nematodes have a simple body plan of a tube-within-a-tube but have survived successfully for the last 600 million years. Most of them are free-living occupying all habitable aquatic and terrestrial habitats, but others have developed relationships with other animals and plants from commensalism/phoresis to parasitism. A large number of genera belonging to several orders of nematodes are known to have colonized animals, from earthworm to man, to feed on their body contents. Lower animals and plants must have been parasitized by nematodes feeding on bacteria, fungi and algae in the Devonian period, some 300 million years ago. Four orders - Tylenchida, Aphelenchida, Dorylaimida and Triplonchida - have evolved a feeding stylet and pumping mechanism to feed on plant and fungal tissues, and the first two orders achieved parasitism of insect and mite haemocoels. Cretaceous must have seen a sudden rise of angiosperms, birds and mammals which were then invaded and successfully parasitized by many groups of nematodes. Ancient saprobiont Rhabditida and Oxyurida-Drilonematida complexes must have been highly adaptive and given rise to modern parasites of higher plants and vertebrates. Phylogenetic studies on these parasites must be based on the analyses of their morphological, molecular, biochemical, cytogenetic, physiological, ecological, behavioral and biogeographical data as well as on their adaptive radiations and survival strategies including cryptobiosis and aging.

NOVEL APPROACH TO THE DISCOVERY OF PARASITISM GENES IN THE POTATO CYST NEMATODE. **G. SMANT (1), Q. Ling (1), P. Prins (1), A. Goverse (1), A. Schots (2), J. T. Jones (3), J. Helder (1), and J. Bakker (1).** (1) Lab. Nematology and (2) Laboratory of Molecular Recognition and Antibody Technology, Wageningen Univ. and Research Centre, PO-box 8123 Wageningen, NL6700ES the Netherlands; (3) Mycology, Bacteriology, and Nematology Unit, SCRI, Invergowrie, Dundee DD2 5DA, UK.

Expressed sequence tags have proven to be efficient tools to identify genes involved in parasitism of plant-parasitic nematodes. This identification depends on the presence of informative homologues in databases. Similarly, cDNA-AFLP has proven to be an efficient procedure to analyze expression patterns in these parasites. Based on a specific expression pattern, genes involved in parasitism can be selected from thousands of transcripts. This selection uses the endonuclease restriction sites and the fragment length as a unique identifier and can, therefore, be made without knowledge of the complete DNA sequence. We have developed computer software, GenEST, which enables us to make a bidirectional link in silico between the information captured in EST databases and the expression profiles produced by cDNA-AFLP. The software generates virtual transcript-derived-fragments from ESTs and compares them with the ones that are displayed on gels. Examples are given to illustrate the power of the software in finding candidate parasitism genes among ESTs of the potato cyst nematode including 'pioneering' sequences.

NEW TECHNOLOGIES FOR INTEGRATED MANAGEMENT OF THE SUGAR BEET NEMATODE, *HETERODERA SCHACHTII*. **H. J. SMITH (1), F. A. Gray (1), D. W. Koch (1), J. M. Krall (1), and L. J. Held (2).** (1) Univ. of Wyoming, Dept. Plant Sciences; (2) Univ. of Wyoming, Dept. Agric. & Applied Econ.

The sugar beet nematode (SBN), *Heterodera schachtii* (Hs), is a major root parasite of sugar beet worldwide. In the U.S.A., sugar beets have been grown for over 60 years in most areas. Losses can be severe, particularly where beets are grown in short rotations. Soil fumigation is usually necessary to produce the needed beet tonnage for sugar refinery operation. SBN-resistant radish and mustard varieties have proven effective to trapping soil populations of Hs and increasing beet yields when second-cropped following harvest of malting barley in Wyoming. Preliminary field tests of the SBN-resistant, intraspecific sugar beet hybrid Nematop shows promise for Wyoming growers. Both trap crops and Nematop lower soil populations of Hs. Also, grid sampling of fields provides necessary information for variable rate

application of Telone II soil fumigant. When sugar beets are grown in a short rotation, integration of these new technologies with crop rotation will provide a safe and effective method of SBN control. Economics of these new technologies is also being studied.

DIVERSITY OF BENEFICIAL TRAITS AMONG ISOLATES OF *STEINERNEMA CARPOCAPSAE*. N. SOMA-SEKHAR and P. S. Grewal. Dept. Entomology, Ohio State University, OARDC, Wooster, OH 44691.

Entomopathogenic nematodes (EPNs) show great promise as biological alternatives to chemicals for control of greenhouse and soil pests. Despite tremendous advances in mass production and application technology, expanding their application to field crops is hampered by susceptibility to abiotic stresses. Genetic improvement of EPNs has been proposed as a means for overcoming this limitation. Extensive survey and characterization of diversity of target traits in natural populations is necessary for a sound genetic improvement program. We evaluated 14 new isolates of *Steinernema carpocapsae* for temperature, desiccation, UV and hypoxia tolerance, virulence and progeny production in comparison to the commercial strain (*S. carpocapsae* ALL). Significant diversity was found among isolates for these traits. Some isolates were found to be superior to the commercial strain in several traits, and one, KMD-33 was superior to the commercial strain in all the traits evaluated. The significant phenotypic differences in beneficial traits observed in this study suggest the feasibility of using these isolates for genetic improvement of *S. carpocapsae* through systematic selective breeding program.

NOTES ON HOST SPECIFICITY AND TAXONOMY IN HETERODERIDAE. D. STURHAN. Biologische Bundesanstalt, Toppeheideweg 88, 48161 Muenster, Germany.

The complex host-parasite relationships in cyst nematodes and related taxa in the family Heteroderidae appear to be largely a result of an evolution process, of co-evolution or parallel evolution of the nematodes and their hosts. Related mono- or oligophagous heteroderids are in general found on related host taxa, on which commonly an adaptive radiation and speciation of the parasites took place. Similarly to morphological characters, a careful analysis of host specificity can serve to indicate incorrect taxonomical placements, to detect relationships and to test or refine phylogenetic hypotheses. Examples are presented where consideration of host specificity contributes to solving taxonomic problems in the *Heterodera avenae* and *H. bifenestra* species groups, the genus *Cactodera*, the *H. humuli* group specialized to Urticaceae and *Heterodera* species parasitising the related Salicaceae, for species complexes in the genus *Globodera* and for species of non-cyst forming heteroderids. Incomplete knowledge of individual host ranges and incorrect data about hosts, however, restrict the utilization of host specificity as a taxonomic tool.

EVOLUTION OF INSECT PARASITISM IN RHABDITID AND DIPLOGASTRID NEMATODES. W. SUDHAUS. Department of Zoology, Free University, 14195 Berlin, Germany.

Parasitism of nematodes in insects arose independently since silurian times in more than 20 lineages. We exclude entoecy, entomopathogeny and necromeny and use the term parasitism only for nematodes that obtain nutrients from a living host. Therefore, many published examples of "parasitic" Rhabditida are classified anew. A short overview is given on the different modes of parasitic life of nematodes in insects: 1) with a change between free-living stages and parasitic larval stages only or 2) parasitism in later stages including adults or only as adults; 3) insects used as paratenic or intermediate hosts for parasites in vertebrates; 4) without a clear-cut distinction between entoecy and parasitism. A diagram depicts the evolutionary steps toward these different parasitic types. The evolution of a dauer juvenile in the Secernentea and special adaptations as inhabitants of patchily distributed habitats formed by decaying material are prerequisites for parasitism. Internal phoresy was a crucial step. From knowledge of the life cycles of parasitic and closely related free-living species associated with insects hypotheses are formulated about how obligate parasites have evolved.

MOLECULAR DIAGNOSTICS OF ECONOMICALLY IMPORTANT NEMATODES. A. L. SZALANSKI, P. G. Mullin, and T. O. Powers. Dept. Plant Pathology, Univ. Nebraska-Lincoln.

Molecular methods for identification of nematode species has become an increasingly active area of research. Phytosanitary regulations for the import and export of commodities requires the identification of numerous nematode taxa. Genetic markers such as nuclear rDNA, and portions of the mtDNA genome, have been applied to many difficult diagnostic problems in Nematology. These studies demonstrate that molecular markers can separate morphologically indistinguishable nematode species. There is, however, a need to demonstrate that these methods are robust and applicable across the entire geographic range of the species. Morphological and molecular vouchers can be made available using digital images, and the Internet. Before regulatory agencies adopt these methods, there must be reasonable assurance that the assays will function with field collected specimens and that the nematodes were correctly identified. Furthermore, false positives must be avoided. Examples will include root-lesion, root-knot, cyst, and seed gall nematodes.

VIRULENCE MECHANISM OF THE RHABDITID NEMATODE *PHASMARHABDITIS HERMAPHRODITA* AND IT ASSOCIATED BACTERIUM *MORAXELLA OSLOENSIS* TO THE GREY GARDEN SLUG *DEROCERAS RETICULATUM*. **L. TAN and P. S. Grewal**. Department of Entomology, The Ohio State University, OARDC, Wooster, OH 44691.

The rhabditid nematode, *Phasmarhabditis hermaphrodita*, has proven to be the most effective biocontrol agent for the grey garden slug *Deroceras reticulatum*. The nematodes invade *D. reticulatum* within 8-16 hr following external exposure, and the posterior mantle region containing shell cavity serves as the main portal of entry for the nematodes. We discovered that bacteria *Moraxella osloensis* associated with the nematodes were pathogenic to *D. reticulatum* if injected into the shell cavity or haemocoel of the slugs. Moreover, the bacteria from 60 hr culture were much more pathogenic than the bacteria from 40 hr culture as indicated with the higher and more rapid mortality of slugs. Axenic first-stage juveniles of *P. hermaphrodita* were not pathogenic to the slug but monoxenic culture of the same stage of nematodes did cause the death of the slug when injected into the shell cavity. Further work suggested that reduction and loss of pathogenicity of the aged dauer juveniles of *P. hermaphrodita* to *D. reticulatum* resulted from the loss of *M. osloensis* inside the nematodes. In conclusion, *P. hermaphrodita* acts only as a vector to transport its associated bacterium *M. osloensis* into the shell cavity of *D. reticulatum* and the bacterium appears to be the sole killing agent. The identification of the toxic metabolites produced by *M. osloensis* is being pursued.

RED FOOD COLORING STAIN: A NEW, SAFER PROCEDURE FOR STAINING NEMATODES IN ROOTS AND EGG MASSES ON ROOT SURFACES. **J. A. THIES**. U.S. Vegetable Laboratory, USDA, ARS, Charleston, SC.

Acid fuchsin and phloxine B are commonly used to stain nematodes in roots and egg masses on root surfaces, respectively. Both stains may be harmful to the user and environment, and require costly waste disposal procedures. We developed safer methods to replace both stains using McCormick Schilling red food color (RFC). *Staining nematodes in root tissues*: Roots were cleared with NaOCl, stained with a 12.5% solution of McCormick Schilling red food color (RFC), and destained briefly in acidified glycerin. Eggs, juveniles, and adult nematodes stained with RFC were equally as visible as those stained with acid fuchsin. *Staining egg masses on root surfaces*: Egg masses of *Meloidogyne incognita* on root surfaces were stained with a 20% solution of RFC for 15 minutes and rinsed in tap H₂O. Egg masses stained with RFC appeared as bright red spheres on the root surfaces that were highly visible even without magnification. Replacement of acid fuchsin and phloxine B with RFC for staining nematodes in root tissue and egg masses on root surfaces, respectively, is safer for the user and for the environment, and also eliminates the costly waste disposal of used stain solutions.

UTILIZATION OF GIS/GPS BASED INFORMATION TECHNOLOGY IN COMMERCIAL CROP DECISION MAKING IN CALIFORNIA. **C. S. THOMAS (1), P. W. Skinner (2), A. Fox (3), and C. A. Greer (1)**. (1) Field Wise, Inc., Bodega Bay, CA; (2) Terra Spase, St. Helena, CA 94574; Fox Weather, Oxnard, CA 93035.

Ground-based weather/plant measurements and imagery were georeferenced in GIS software using an integrated approach to determine insect and disease risk and crop cultural requirements. Weather forecasts and disease weather forecasts for agricultural areas were constructed with elevation, weather and satellite data. Models for 6 pests and 12 diseases for various crops were calculated and presented daily in georeferenced maps for agricultural areas in N. CA and WA. Grape harvest dates/yields were also predicted with high accuracy. The data generated from the GIS/GPS analyses were used to make management decisions over a large number of acres in the western US. Information is distributed daily over the Internet as regional weather and disease risk maps. Site specific weather and disease risk data is available for a fee. Use GIS/GPS technology to analyze data, semiautomated, is discussed.

MELOIDOGYNE PARTITYLA: AN EMERGING NEMATODE PEST OF PECAN IN NEW MEXICO. **S. H. THOMAS (1), J. M. Fuchs (1), and A. L. Jacobson (1)**. (1) Dept. of Entomology, Plant Pathology, and Weed Science, New Mexico State University, Las Cruces 88003.

Meloidogyne partityla was described from pecan in South Africa in 1986, and reported in Texas in 1996. In 1995 declining pecans in NM were found to be infested with root-knot nematodes. These trees failed to respond to normal fertilization and irrigation practices. Symptoms were most apparent on mature trees in sandy soil, and included die-back in the upper canopy, chlorotic foliage, and numerous small galls on feeder roots. All attempts to culture the nematode on tomato have been unsuccessful. In 2000 this nematode was confirmed as *M. partityla*. We have solicited specimens from pecans around the USA since 1998 for mtDNA fragment comparison with known populations of *M. partityla*. Nucleotide sequence comparisons among samples from NM, TX and AZ are being used to identify restriction enzyme sites that differentiate *M. partityla* from other root-knot species. In a related management study, no differences in nematode populations occurred after treatment with 7.5 kg a.i./ha aldicarb for two seasons. Further study is needed to determine the distribution and pathogenicity of *M. partityla* in pecan.

NEMATODE REPRODUCTION IN TALL FESCUE INFECTED WITH DIFFERENT ENDOPHYTE STRAINS. **P. TIMPER (1), R. N. Gates (1), and J. H. Bouton (2).** (1) USDA-ARS, P.O. Box 748, Tifton, GA 31793; (2) Dept. Crop and Soil Science, Univ. of Georgia, Athens, GA 30602.

Tall fescue (*Festuca arundinacea*) infected with its native fungal endophyte (*Neotyphodium coenophialum*) is more drought tolerant and resistant to pests, including nematodes, than fescue without the endophyte. However, the native endophyte produces ergot alkaloids which are toxic to grazing animals. Recently, tall fescue cultivars have been infected with non-toxic endophyte strains. Our objective was to determine whether fescue infected with these non-toxic strains is resistant to lesion nematodes, *Pratylenchus* spp. In a greenhouse experiment, nematode reproduction was compared in two fescue cultivars infected with the native strain, with two non-toxic strains (AR542 and AR584), and endophyte-free fescue. The number of nematodes per root system was lower in plants infected with the native strain (24) than in plants infected with strains AR542 (118), AR584 (111), and endophyte-free plants (146). Therefore, the two non-toxic strains, unlike the native strain, do not confer resistance to lesion nematodes. Additional non-toxic strains should be tested for nematode resistance.

IDENTIFICATION OF *MELOIDOGYNE INCOGNITA* GENES BY DIFFERENTIAL DISPLAY ON INFECTED ROOTS. **T. TYTGAT (1), I. Vercauteren (2), J. De Meutter (2), G. Gheysen (3), and A. Coomans (1).** (1) Institute of Zoology; (2) Dept. of Plant Genetics; (3) Faculty of Agriculture and Applied Biological Sciences, Universiteit Gent, K.L. Ledeganckstraat 35, B-9000 Gent, Belgium.

Arabidopsis thaliana plants were inoculated with freshly hatched *Meloidogyne incognita* J2 juveniles. Root galls were harvested 2, 3, 4, 5 and 7 days post inoculation. Total RNA was isolated from the root galls and non-infected root tissue. After first strand synthesis, differential display analysis was performed with random decamers. 29 bands specific for gall tissue were isolated, reamplified and cloned. DNA sequence analysis revealed 75 different sequences. By southern blotting on genomic DNA, 6 clones were identified to be from nematode origin. None of them showed homology to any known gene sequence in the available databases. Currently, full length clones are isolated from a cDNA library, and in situ hybridisation is performed.

STRAINS FROM THE *BURKHOLDERIA CEPACIA* COMPLEX: RELATIONSHIP TO OPPORTUNISTIC PATHOGENS. **P. A. R. VANDAMME (1) and E. Mahenthiralingam (2).** (1) Laboratorium voor Farmaceutische Microbiologie, Universiteit Gent, Belgium; (2) Cardiff School of Biosciences, Cardiff University, UK.

B. cepacia is a versatile organism with soil, water, animals, plants and humans as niches. Although first known as a plant pathogen, it is now recognized as a useful organism with many biotechnological applications. It simultaneously has become notorious as a life-threatening pathogen in immune suppressed human hosts. During the past decade, a polyphasic taxonomic study including comparative 16S rDNA and RecA sequence analysis, DNA-DNA hybridization, whole-cell protein and fatty acid analyses, various DNA fingerprinting methods, and biochemical characterization, was performed on over 2500 *B. cepacia* like isolates. This study revealed the presence of various organisms that are regularly misidentified as *B. cepacia*. It also demonstrated that *B. cepacia* is a complex of at least nine closely related species, which are all present in environmental and human samples. Candidates for biotechnological applications occur in most of these species. However, there are several salient differences between the members of the *B. cepacia* complex.

A PUTATIVE HEAT-STABLE NEMATODE RESISTANCE GENE *MI-9* FROM *LYCOPERSICON PERUVIANUM* IS CONSTITUTIVELY EXPRESSED IN LEAVES AND ROOTS. **J. C. VEREMIS, I. Kaloshian, and P. A. Roberts.** Department of Nematology, University of California, Riverside, CA 92521.

All tomato cultivars resistant to root-knot nematodes (*Meloidogyne* spp.) rely on a single gene *Mi-1*, located on chromosome 6, that is heat-unstable. We have identified heat-stable resistance to root-knot nematodes in wild tomato, *Lycopersicon peruvianum* accession LA2157. The inheritance of the single dominant gene *Mi-9* from the northern wild tomato accession LA2157 was evaluated in segregating progenies. Mapping determined the linkage of *Mi-9* with markers on chromosome 6. Homology of the *Mi-9* locus from LA2157 and the *Mi-1* locus was investigated with *Mi-1* specific molecular markers. Using primers designed to amplify *Mi-1*, a DNA fragment was amplified that cosegregates with *Mi-9*. Molecular markers were designed only for the *Mi-9* locus. Further, using primers of *Mi-1.2* in RT-PCR of root and leaf cDNAs, the putative *Mi-9* gene was constitutively expressed in roots and leaves, further indicating the homology to *Mi-1* gene. Genetic and molecular characterization of *Mi-9* will clarify its relationship to *Mi-1* and to the other host plant resistance genes.

PLANT-PARASITIC NEMATODES ASSOCIATED WITH HORSERADISH IN ILLINOIS. **S. A. WALTERS (1), M. Babadoost (2), J. P. Bond (1), and D. I. Edwards.** (1) Dept. of Plant, Soil and General Agriculture, Southern Illinois University, Carbondale, IL 62901; (2) Dept. of Crop Sciences, University of Illinois, Urbana, IL 61801.

Approximately, half of the total commercial horseradish (*Amoracia rusticana* Gaertn., Mey., Scherb.) in the United States is produced in Mississippi River Valley, near East St. Louis, Illinois. Surveys were conducted during 1999 and 2000

to determine the plant-parasitic nematodes associated with horseradish in this area by assaying soil and root samples collected from the commercial fields. Five genera of plant-parasitic nematodes, *Pratylenchus*, *Tylenchorhynchus*, *Helicotylenchus*, *Xiphinema*, and *Hoplolaimus*, were identified in the extractions from the soil samples. Plant-parasitic nematodes extracted from the root samples belonged to the genera *Pratylenchus*, *Tylenchorhynchus*, and *Helicotylenchus*. Populations of the extracted nematodes from soil and root samples were low and it appears that plant-parasitic nematodes do not significantly affect horseradish production in Illinois.

MULTIPLE CROPPING SYSTEMS FOR NEMATODE MANAGEMENT. K.-H. WANG (1) and R. McSorley (1). (1) Dept. Entomology and Nematology, University of Florida, Gainesville, FL 32611.

The design of multiple cropping systems and appropriate crop sequences are useful in nematode management. The individual crops in cropping systems may be arranged 1) in time, such as in crop rotation between cash crop and winter or summer cover crop, in continuous monoculture, or in fallowing between cash crop cycles; 2) in space, such as intercropping; or 3) in combinations of time and space. The planning of crop sequences over time has been used much more often than intercropping for nematode management. Management of cyst, root-knot and other nematodes by cropping systems were reviewed. The success of the system relies on detailed biological information on nematode genotypes, crop germplasm, relationship between nematode density and crop yield, nematode biology, host range, population dynamics, and the economics of management practices. Environmental, seasonal, and regional effects as well as potential interactions with other organisms should be considerate. Integration of multiple-cropping practices with other nematode management methods should also be evaluated. Unfortunately, much of this information is highly site specific.

COVER CROPS FOR ROTYLENCHULUS RENIFORMIS MANAGEMENT ON PINEAPPLE. K.-H. WANG, B. S. Sipes, and D. P. Schmitt. Dept. of Plant and Environmental Protection Sciences.

Crotalaria juncea, *Brassica napus*, and *Tagetes erecta* were evaluated for their potential as cover crops for *Rotylenchulus reniformis* management in intercycle and intercrop systems. In an intercycle system, *C. juncea* suppressed *R. reniformis* populations on pineapple most effectively among the three crops with suppressive effect comparable to 1,3-dichloropropene (1,3-d). In an intercrop system, *C. juncea* and *B. napus* suppressed *R. reniformis* population development better than *T. erecta*, weeds, or pineapple. Population densities of *R. reniformis* remained low in *C. juncea* plots for 3 months after pineapple planting. In a series of greenhouse tests, *C. juncea* delayed *R. reniformis* female development, and produced allelopathic compounds in its leaf leachate suppressive to *R. reniformis* mobility. Soil amended with *C. juncea* increased the population densities of nematode-trapping fungi in several soils except those recently treated with 1,3-d. Thus, planting of *C. juncea* is a good supplemental *R. reniformis* management practice.

TO BE OR NOT TO BE, A SYMBIONT. J. M. WEBSTER (1) and K. Walsh (1). Simon Fraser University.

There is a mutualistic relationship between entomopathogenic nematodes (EPNs) and some bacterial species but not with others in EPN infected insect cadavers. When *Galleria mellonella* larvae were infected with infective juveniles (IJs) of *Steinernema feltiae* (Rstrain) populations of *Xenorhabdus* sp. reached a peak at 96h postinfection and of *Acinetobacter* sp. soon after. Both bacterial species originated from the IJs. Inhibition bioassays of the antimicrobial activity of the secondary metabolites of *Xenorhabdus* and *Acinetobacter* showed only slight activity (0.5mm) against the reciprocal bacterial species but the metabolites of both species showed strong activity (3.8mm) against *Enterococcus* sp., a bacterium that originated from the gut of the *G. mellonella* larvae. *Steinernema feltiae* reproduced strongly on its *Xenorhabdus* symbiont and on other *Xenorhabdus* species but only weakly on *Acinetobacter* and *Enterococcus* in *G. mellonella* larvae.

ARE BT TOXINS NEMATICIDES? J.-Z. WEI (1), K. Hale (1), L. K. Carta (2), and R. V. Aroian (1). (1) Section of Cell and Developmental Biology, Division of Biology, University of California, San Diego, La Jolla, CA 92093-0349; (2) USDA-ARS, Nematology Lab, Beltsville, MD 20705-2350.

Bacillus thuringiensis (*Bt*) delta-endotoxins, a family of crystal (Cry) proteins, are widely used as insecticides in agriculture. Recently, we described that *Bt* toxins may be also effective nematocides. To more fully address the potential of Cry toxins as nematocides to control plant-parasitic-nematodes (PPNs), we analyzed the toxicities of eight Cry proteins (Cry 5A, 5B, 6A, 6B, 12A, 13A, 14A, and 21A) on five free-living nematodes (*C. elegans*, *Pristionchus pacificus*, *Panagrellus redivivus*, *Acrobeloides*, and *Distolabrellus veechi*). The results of general toxicity assays, morphology, and brood size indicated that indeed some of these Cry proteins are potent nematocidal toxins. However, some had little effect on nematodes. One nematode species was resistant to all toxins. We found that one *Bt* toxin could be trimmed to a small 42 kD active core that was toxic to almost all these diverse nematodes. We believe that *Bt* toxins show good potential to control PPNs.

CONTROL OF SOYBEAN CYST NEMATODE HETERODERA GLYCINES WITH LIME-STABILIZED MUNICIPAL BIOSOLIDS. T. W. WELACKY (1) and E. Topp (2). (1) Agriculture and Agri-Food Canada, GPCRC, Harrow, ON. N0R 1G0; (2) Agriculture and Agri-Food Canada, SCPFRC, London, ON. N5V 4T3.

The objective of this study was to determine if commercially prepared lime-stabilized sludge, licenced as an agricultural soil conditioner (N-Viro Energy Systems, Ltd.; U.S. Patent 4,902,431), suppresses SCN. In a 3-year experiment, field plots cropped to susceptible soybeans received 2.2 dry tonnes/hectare of mushroom compost, municipal biosolids treated with either cement kiln dust or coal combustion ashes as the liming agent, calcitic lime or no treatment. In comparison with the control treatments, the alkaline treated soil conditioners reduced SCN populations, but not significantly ($P < 0.05$). However, in parallel bench-scale pot studies, biosolids stabilized with either cement kiln dust or coal ashes significantly suppressed SCN, at application rates ranging from 2-20 tonnes/hectare. Suppression was evidenced by lower cyst and egg counts in soil experimentally infested with cysts, and planted to soybeans. This study has established that lime-stabilized biosolids can suppress SCN, but further work is required to establish under what conditions it could be effective in commercial farming situation.

REDUCTION OF SOIL POPULATIONS OF SOYBEAN CYST NEMATODE *HETERODERA GLYCINES* BY CROP ROTATION WITH NON-HOSTS AND VARIETAL RESISTANCE. T. W. WELACKY (1), T. R. Anderson (1), A. U. Tenuta (2). (1) Agriculture and Agri-Food Canada, GPCRC, Harrow, ON. N0R 1G0; (2) Ontario Ministry of Agriculture, Food and Rural Affairs, P.O. Box 400, Ridgetown College, Ridgetown, ON, Canada N0L 2C0.

Long term rotation plots were established in 21 soybean producer fields in Ontario, Canada in 1994. Treatments of resistant, Bell and susceptible, Elgin 87 soybeans, and a continuously fallow treatment. Plots were sampled annually for soil populations of cysts and eggs at planting and harvest. Crop rotation sequences of 1, 2, or 3 year rotations with commercial non host crops. Soil types ranged from coarse sand to fine sandy silt loam. In 1994 SCN soil population levels averaged 13,600, 14,115 and 16,003 eggs/100 g dry soil in the resistant, fallow and susceptible plots, respectively. In 2000, SCN populations decreased significantly ($P < 0.05$) for each treatment to 2,705, 1,611 and 7,928 eggs/100 g soil, respectively. Soil populations declined consistently with resistant and fallow treatments compared to treatments with susceptible soybeans. Similar trends were observed in the number of eggs/cyst with each treatment, from 65, 61 and 79 to 13, 11 and 32, respectively.

CHANGES IN LIPID CONTENT OF SOYBEAN CYST NEMATODE JUVENILES. J. M. Wells (1), M. N. Lacouture (1), and P. M. TEFFT (1). (1) Biology Department, St. Joseph's University.

A computerized morphometric program was used to estimate lipid stores in second stage juveniles of the soybean cyst nematode (SCN) that were stained with oil red O stain. The area and number of lipid containing globules were measured from digitized images. The amount of neutral fat in SCN juveniles varied greatly. No relationship was found between the amount of lipid in nematodes and time after hatching from 0-6 days. Only about 20% of the nematodes remained viable after 6 days. Lipid content was reduced in juveniles that were hatched from eggs collected from cysts that were stored in tap water form 1-9 weeks. The total neutral fat content was reduced by 50% over a nine week period. Both the total area of lipid containing globules decreased per worm as well as the average area per vesicle. Results of this study indicate that lipid utilization and/or bioconversion of lipid occurs in the pre-hatch juveniles of SCN.

POPULATION FLUCTUATIONS OF LESION NEMATODE, *PRATYLENCHUS VULNUS*, AND RING NEMATODE, *CRICONEMELLA XENOPLAX* IN CALIFORNIA WALNUT ORCHARDS. B. B. WESTERDAHL (1), W. O. Reil (2), and C. E. Anderson (1). (1) Dept. Nematology, University of California (UC), Davis, CA 95616; (2) UC Cooperative Extension, Woodland, CA 95695.

Three sprinkler irrigated orchards in Solano County were sampled at monthly intervals over a three year period. Four samples were taken per orchard with a trenching shovel at a depth of 0 to 60 cm where feeder roots were found to be most abundant. Lesion and ring nematodes were recovered from soil via elutriation followed by sugar centrifugation. Feeder roots were weighed and placed in a mist chamber for extraction of lesion nematode. Lesion nematode populations in soil were lowest in December and highest in March and August. Populations within roots were lowest December through March and highest in October. Ring nematode populations in the same orchards were highest in May, June and July, corresponding to time of highest feeder root biomass in samples, and lowest in August, October and December. This information combined with the finding that feeder root biomass is lowest in March could lead to improved treatment timings for nematode control measures not expected to affect nematodes within roots.

OPTIMIZING TIME OF YEAR FOR REMOTE SENSING BASED NEMATODE SAMPLING IN COTTON. T. A. WHEELER (1), K. Siders (2), and H. W. Kaufman (1). (1) Texas A&M Research and Extension Center, Lubbock, TX; (2) TAEX, 1212 Houston St., Levelland, TX 79336.

Six cotton fields were photographed from an airplane using color infrared film on 20 June, 10 July, 4 August, 22 August, and 13 September, 2000. These fields were sampled for nematodes in November 2000 based on differences in soil type or plant vigor as seen with the images. Each field (24 to 48 ha) had 3 to 5 composite soil samples taken. Root-knot nematode was present at low to moderate levels in all six fields. In two of the fields there was no correlation between

root-knot nematode density and reflectance from any images. In three fields there was a correlation between root-knot nematode density and reflectance from an image taken on 10 July. Reflectance from the green band was correlated to nematode density in these fields. In one field there was a correlation between $\log_{10}(\text{root-knot nematode density}+1)$ and reflectance from the red/green bands taken on 20 June. Fall root-knot nematode densities as low as 100/500 cm³ soil could be correlated with reflectance patterns.

GENOME PROJECTS FOR HUMAN NEMATODE PARASITES. **S. A. WILLIAMS.** Dept. Biological Sciences, Smith College, Northampton, MA 01063 and Molecular and Cellular Biology, University of Massachusetts, Amherst, MA 01003.

Lymphatic filariasis (LF) is caused by the nematodes *Brugia malayi* and *Wuchereria bancrofti*. These parasites infect 130 million people worldwide: 44 million are incapacitated or disfigured by the disease and 76 million have damage to their renal/lymphatic systems. LF has recently been recognized as the second leading cause of permanent/long-term disability worldwide. A related filarial nematode, *Onchocerca volvulus*, is the causative agent of river blindness and infects 18 million people living in sub-Saharan Africa. The Filarial Genome Project was launched in 1994 to study the genome of *B. malayi* while the River Blindness Genome Project was organized in 1997 to study the genome of *O. volvulus*. The major goal of these two genome projects has been the identification of new drug targets and vaccine candidates using EST-based gene discovery. To this end, 22,000 ESTs have been sequenced from *B. malayi* (8,000 genes identified) and 14,000 from *O. volvulus* (5,000 genes identified). These data provide a vast resource that will assist in the identification of new targets for chemotherapy and for the development of vaccines.

TOMATO RESISTANCE GENES AGAINST ROOT-KNOT NEMATODES AND APHIDS. **V. M. WILLIAMSON, S. K. Seah, C. F. Hwang, R. L. Wrobel, and J. Yaghoobi.** CEPRAP, University of California, Davis, CA 95616.

The tomato gene *Mi-1* confers resistance against several species of root-knot nematodes and to specific isolates of potato aphid. This gene encodes a protein that belongs to the NB-LRR class of resistance genes. The *Mi-1* region of the genome carries two clusters of *Mi-1* homologs on chromosome 6 within the chromosomal region that was introgressed from the wild species *Lycopersicon peruvianum*. One cluster carries the functional nematode resistance copy, *Mi-1.2*, an expressed homolog, *Mi-1.1*, and a pseudogene, *Mi-1.3*. The other cluster carries at least four additional homologs. There are at least 8 homologs in the corresponding chromosomal locations in susceptible tomato and at least two homologs on a different chromosome. DNA blots indicate that homologous sequences are present in other Solanaceous plant species. Structural and function analysis of *Mi* and its homologs is in progress. The phenotypes of domain swaps and single nucleotide changes between *Mi-1.1* and *Mi-1.2* are being characterized to dissect the mechanisms of signal transduction mediated by *Mi*.

EFFECTS OF DISSOLUTION OF GRASS CUTICLE LAYERS. **P. T. WRIGHT (1).** (1) Dept. Soil Science, The University of Western Australia, 35 Stirling Hwy, Crawley, Western Australia, 6009.

Dissolution of foliar cuticle in couch *Cynodon dactylon* and bent *Agrostis stolonifera* grass treated with "Citowett" (ai: Alkylaryl polyglycol Ether). The aim of the treatment was to eradicate couch with minimum disruption to bent on a golf green. Citowett was used to dissolve cuticle layers to permit absorption and allow maximum surface contact of a herbicide/plant growth regulator mix consisting of Starane (Fluroxypyr) and Primo (Trinexapac-ethyl). Results of this investigation show: 1. Increased sensitivity of the target grass to the herbicide/plant growth regulator mix. 2. Relatively less sensitivity of bent. 3. Although there was extensive removal of cuticle by the Citowett treatment, the level of dissolution was not adequate to allow an increase in invasion of the leaves by foliar pathogens or pests.

MORPHOLOGICAL AND MOLECULAR TAXONOMY OF *LONGIDORUS* OCCURRING IN ARKANSAS. **W. YE and R. T. Robbins.** Dept. Plant Pathology, University of Arkansas.

About 700 soil samples were collected from 32 Arkansas counties in a survey from natural plants in 1999-2000. Nematodes were extracted from samples by combining the roiling-sieving and sugar centrifugation methods. *Longidorus* was found in 196 samples and the most frequently encountered species was *L. diadecturus*, and a few males were found for the first time. *Longidorus elongatus*, *L. breviannulatus*, *L. fragilis*, *L. crassus* also were identified. One hundred populations from 23 species obtained from all over the world were compared with the Arkansas specimens. Several new species also were found in these samples. Discriminating between *Longidorus* species is very difficult because their diagnostic morphometric characters often greatly overlap. A PCR assay with ribosomal DNA primers derived from the ITS region has been developed and intraspecific polymorphism was present and distinguishable by RFLP. Cloning and sequencing of amplified products in order to distinguish the species and examine their relationships is still in progress.

A DATABASE MANAGEMENT SYSTEM FOR NEMATODE TAXONOMISTS. **W. YE and R. T. Robbins.** Dept. Plant Pathology, University of Arkansas.

This study presents a relational database management system for the purpose of storing data necessary for nematode taxonomists. The system was developed using Microsoft Access 2000, and consists of two independent databases: (1)

Nematode sample and (2) Taxonomic literature. The sample database includes information on the survey number, plant, locality, sample date, identification result (slide number, genus, species, density), collector (name, title, birthday, gender, address, email, photo, publication), and slide collections (genus, species, life stage, number). The taxonomic literature database contained 3,322 research papers, reviews, and books in the field of plant nematode taxonomy, morphology, surveys, techniques and molecular biology collected by the first author. Information of each paper includes author, publication year, title, journal name, volume, issue number, starting page, ending page, genus and species involved, description status. This system is useful for data integrity, fast information retrieval and flexible operation on nematode sample, identification result, slide collection, and literature query.

COMPARISON OF TWO METHODS TO EVALUATE SOYBEAN FOR RESISTANCE TO SOYBEAN CYST NEMATODE. L. D. YOUNG. USDA ARS, P.O. Box 345, Stoneville, MS 38776-0345.

Two methods of evaluating soybean for resistance to the soybean cyst nematode, *Heterodera glycines* Ichinohe, were compared from 1996 to 1998 using entries in the University of Tennessee yield trials. Method 1 involved growing seven plants, each in a separate pot, of each entry in soil infested with 1,000 eggs of the nematode for approximately 30 days. Roots of each plant were exposed by crumbling the soil ball, and the number of nematodes present was visually estimated into one of five classes. A weighted average was calculated for each entry. Method 2 involved growing five replications per entry in infested soil for 35 days, extracting the females from the roots and soil, and counting the females with a microscope. Mean number of females was expressed as a percentage of females extracted from a standard susceptible cultivar. Entries were tested separately for reaction to races 3 and 14. Regression analysis showed good correlation between methods; however, method 1 did not allow consistent separation of entries into four standard classes of reaction, particularly with race 14. Method 2 is preferred for dissemination of information to farmers unless required labor prevents its use.

CHANGES IN NEMATODE COMMUNITY STRUCTURE AFTER THE APPLICATION OF PLANT AMENDMENTS. I. A. ZASADA and H. Ferris. Department of Nematology, University of California, Davis, CA 95616.

We have evaluated plant amendments, proven to suppress plant-parasitic nematode populations, on soil nematode communities. Levels of *Meloidogyne javanica* were lowest in soils amended with garlic and English ivy, but were not affected by onion or pumpkin. Nematode faunal diagrams provided graphic representation of changes in the soil nematode community. Garlic alone suppressed faunal diversity and the abundance of taxa purportedly intolerant of disturbance. Faunal analysis indicated progression of the soil food web toward a basal condition, with Cephalobidae dominating. Conversely, ivy, onion and pumpkin maintained diversity of the soil food web. Soon after application, enrichment of the soil food web was reflected by an increase in cp-1 taxa. Several months later disturbance-intolerant nematodes dominated the nematode community. We conclude that degradation products of these plant amendments have different affects on the soil nematode community. Some behave as biocides and others are more enriching and may allow development of a soil fauna with sufficient structure to create a suppressive environment for plant-parasitic nematodes.

DIGITAL DATABASES FOR TEACHING, RESEARCH, AND EXTENSION. U. ZUNKE (1) and J. D. Eisenback (2). (1) Institute for Applied Botany, University of Hamburg, D02355 Hamburg, Germany; (2) Department of Plant Pathology, Physiology and Weed Science, Virginia Tech, Blacksburg, VA 24061 USA.

Scientific, educational, and research images that are invaluable resources for teaching, making Web pages, Powerpoint presentations, extension publications, brochures, and seminar and classroom lectures have been compiled on CD ROMs. Each image is described with a figure legend and placed in a catalog of thumbnails that is searchable by keywords. Each of the CD ROMs contain most of the images that are needed to teach an introductory course on plant nematology, fungal diseases of plants, and insects that feed on plants. These images are among the best in the world. Since these images are preserved on CD-ROM they will not fade, scratch, or be subjected to other imperfections. The pictures can be used for educational purposes provided proper credit is conferred. Volumes assembled thus far include two volumes related to nematodes, one on fungi that cause plant diseases, and one the contains images of insects that feed on plants. Additional volumes of each series are planned or are currently in production. Submission of photographs for these volumes are welcome.