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ABSTRACTS

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PLANT-PARASITIC NEMATODES ASSOCIATED WITH ONION ROOTS AND SOILS IN NEW YORK STATE. **Abawi, G. S., and J. W. Ludwig.** Dept. of Plant Pathology, NYSAES, Cornell Univ., Geneva, NY 14456.

A survey was conducted in 1998-99 in the onion producing areas of Orange, Oswego, Genesee, Yates and Wayne counties. Composite soil (209) and root (139) samples were collected from 51 fields. Soil and root samples were collected at close to planting and bulbing stage, respectively. Nematodes were extracted from soils (pie-pan) and roots (shaker), and the soil samples were also indexed in the greenhouse by planting them with lettuce. *Meloidogyne hapla* (Mh) was most prevalent on onions, as it was recovered from 51% and 84% of fields when sampled by direct extraction from soil or by the lettuce bioassay, respectively. The population density of Mh was 103 (40-720) juveniles/100 cm^3 soil and 18 (3-72) eggs/g root. The lesion nematode (*Pratylenchus* spp.) was recovered from 24% and 35% of the fields when analyzed by soil and root samples, respectively. The number of lesion nematode was 67 (40-160)/100 cm^3 soil and 289 (2-2940)/g root. Stubby-root, bloat, and stunt nematodes were recovered from 3, 1, and 4 fields, respectively. The recovery of root-knot and lesion nematodes differed greatly among the different production regions.

THE ANTAGONISTIC EFFECT OF NATURAL PRODUCTS ON NEMATODE EMBRYONIC DEVELOPMENT, LARVAL DEVELOPMENT, AND SURVIVAL. **Abdel-Rahman, Fawzia, R. A. Salah El Din, and R. Nsaif.** Biology Department, Texas Southern University, Houston, TX 77004.

Organic and aqueous extracts from wild desert flora, marine algae, soil bacteria, and fungi, were evaluated for their potential nematicidal activities. Extraction was carried out on air-dried plants and algae using a mixture of methanol/methylene chloride (1:1) to obtain the organic fraction. Aqueous extracts were obtained by water extraction of the left over materials from the organic extraction. Nematicidal activity of soil fungi and bacteria were carried out with their metabolites in the culture filtrates. A bioassay was performed on *Meloidogyne incognita* larva and egg-masses, as well as on other genera including *Helicotylenchus, Plectus, Aphelenchus*, and *Mononchus*. A water-screen bioassay was used to investigate the nematicidal activities of the tested natural-products on the embryonic development, egg hatching, and larval and adult survival of *M. incognita*. Larval development of *M. incognita* was monitored on tomato plants after their exposure to several natural products. The results showed that some extracts stopped the embryonic development in different stages, which prevented or lowered egg hatching. Several other natural products demonstrated activity associated with high mortality of the nematode larvae and adults. Additionally they interfered with larval development and inhibited their maturation into adults. Results of our finding will be presented.

TESTING FACTORS THAT PROMOTE NEMATODE SPECIATION. **Adams, Byron J., K. B. Nguyen, and H. L. Smith.** University of Florida, Entomology and Nematology Department, Gainesville, FL 32611-0620.

Nematodes are arguably the most abundant metazoans on earth, and possibly one of the most species-rich. The actual number of nematode species is more controversial, with estimates ranging

from less than 50,000 to over 100,000,000. Hypothetical factors that promote nematode species-richness include their small body size, small geographic and ecological range requirements, facultative utilization of males, accelerated rates of DNA substitution and molecular evolution, and so-called ''key innovations". Alternatively, species-richness may simply be an artifact of variation in speciation and extinction probabilities. By comparing cladogenesis among sister groups in the Nemata, we test several speciation-promoting hypotheses. Our results identify biological factors correlated with nematode species-richness, and can be extended to estimates of nematode biodiversity.

ISOLATION OF GENE TRANSCRIPTS UP- AND DOWN-REGULATED IN GIANT CELLS INDUCED IN HOST ROOTS BY *MELOIDOGYNE JAVANICA.* **Ah-Fong, V., R. H. Potter, J. H. Khong, and M. G. K. Jones.** Western Australian State Agricultural Biotechnology Centre, Murdoch University, Perth, Western Australia, 6150.

The technique of Differential Display RT-PCR has been optimized and used to compare expression of gene transcripts between control root tissue and giant cell enriched tissues of tomato plants infected with *Meloidogyne javanica*. A total of 25 up- or down- differentially expressed bands were identified in 33P labelled autoradiograms using DD-RT PCR. Of these, 12 bands were cloned and sequenced. The expression of 6 was studied in more detail. Using semi-quantitative real time fluorescence RT-PCR, normalized against expression of actin, the expression of five of these genes was studied at four times after infection (5, 15, 25 and 30 days). The results showed that one was highly up-regulated (IC9, 69-fold up-regulated at 15 days), one was strongly down-regulated (HA/HT1, 28-fold down-regulation at 15 days), two showed about 10-fold changes in expression, and one was similar in expression to the control. Significantly, the sixth transcript (IC1) was of nematode origin, although it was routinely isolated from giant cell enriched tissue. If this is not a contaminant it may be the first evidence for a nematode transcript in giant cells.

EFFECT OF THE MANURE ADDITION AND SOIL MOISTURE ON EGG PARASITE FUNGI OF THE SOYBEAN CYST NEMATODE. **Aiba, Satoshi.** Laboratory, National Agriculture Research Center, Kan-nondai 3-1-1, Tsukuba, Ibaraki, Japan, 305-8666.

A survey for fungal egg parasites of the soybean cyst nematode (*Heterodera glycines*, SCN) was conducted in azuki-bean fields, with and without manure fertilization, in Hokkaido (Japan). An unidentified fungal parasite (type A) was commonly detected in both field conditions. Percentages of this fungus isolated from colonized SCN eggs were 49.8 % of all the fungi found in fields without manure and 90.0 % from fields with manure application. An additional experiment was carried out using field soil without manure at high (pF 2.3) and low (pF 1.5) soil moisture conditions and growing soybean for 90 days. The ratio of fungi isolated from colonized eggs at high moisture was similar to that in field, namely type A was 40.2 %, but when soil was dry, *Paecilomyces* sp. became 84.7 % of all the fungi isolated from eggs. These results suggest that the fungus type A may be the main natural enemy of SCN in azuki-bean fields of Hokkaido and is activated by manure. *Paecilomyces* sp. could take its place and became the dominant SCN egg parasite under dry conditions.

PLANT PARASITIC NEMATODES ASSOCIATED WITH TURFGRASS IN AL-QASSIM, SAUDI ARABIA. **Al-Rehiayani, Suloiman,* A. A. Farahat, and M. M. Belal.** Plant Protection Department, King Saud University Al-Qassim Branch, Saudi Arabia.

A plant-parasitic nematode survey on irrigated ornamental turfgrass was made at 25 sites in Al-Qassim area. The sites included ornamental turfgrass commercial farms, soccer fields, and the cities general gardens. Soil and root samples were taken mainly from bermudagrass cv. Tifway and cv. Tifgreen. The root-knot nematodes, *Meloidogyne javanica, M. incognita* and *Meloidogyne* sp., which were found in 92% of the sites sampled, were the major turfgrass pests. Other nematode genera and their frequency of occurrence among all sites were *Tylenchorhynchus* (81%), *Cricone-*

mella (80%), *Ditylenchus* (50%), *Pratylenchus* (48%), *Trichodorus* (32%), *Helicotylenchus* (8%), and *Xiphinema* (8%).

SOYBEAN CYST NEMATODE RESISTANCE GENES IN PI89.772 SOYBEAN. **Assunção, M. S.,[1] G. R. Noel,[1,2] and B. W. Diers.[1]** [1]Department of Crop Sciences, University of Illinois, and [2]USDA, ARS, Urbana, IL 61801.

The number of resistance genes in PI89.772 that confer resistance to *Heterodera glycines* is unknown. Crosses of PI89.772 H >Lee 74 = and PI88.788 H PI89.772 were made in the field and greenhouse. To certify that F_1 plants resulted from a cross rather than a selfing, simple sequence repeat (SSR) analysis was applied to F_1 plants and their parents. Several F_1 and F_2 families from each cross and 98 F_3 families from PI89.772 H Lee 74 and 75 F_3 families from PI88.788 H PI89.772 were tested with an inbred line of *H. glycines* developed on PI88.788. Approximately 4,500 individual plants growing in 250 cm^3 sand were inoculated with 4,000 eggs. Thirty days after inoculation the number of females that developed on each plant was determined. Plants were considered resistant if the number of females that developed was lower than 10% of the females that developed on the susceptible Lee 74 (0–500). Segregation ratios for resistance and susceptibility in F_1 = s, F_2 = s, and F_3 = s indicate that resistance is not conferred by a single dominant gene and suggests the presence of at least one pair of recessive genes.

ANALYSIS OF THE 16S rRNA GENE PROVIDES NEW INSIGHTS ON THE PHYLOGENETIC RELATIONSHIPS AMONG *PASTEURIA* SPP. **Atibalentja, N.,[1] G. R. Noel,[1,2] and L. L. Domier.[1,2]** [1]Department of Crop Sciences, University of Illinois, and [2]USDA, ARS, Urbana, IL 61801.

The criteria (e.g., morphology, life cycle, and host range) currently used to describe species and isolates of *Pasteuria* have proved unsufficient for taxonomic purposes. To assess how recent advances in the molecular biology of *Pasteuria* could assist in resolving the phylogenetic relationships among *Pasteuria* spp., the 1,485-bp sequence of the 16S rDNA from an Illinois *Pasteuria* isolate that parasitizes *Heterodera glycines* was compared to homologous sequences of 32 other bacterial species, including *P. ramosa* and *P. penetrans*, parasitic to water fleas and root-knot nematodes, respectively. Phylogenetic analyses confirmed the position of the genus *Pasteuria* among the *Bacillaceae*, and further revealed that *P. ramosa* diverged before the speciation of nematode-infecting *Pasteuria*, with a dissimilarity index of 7% compared to 2% between *P. penetrans* and the Illinois *Pasteuria*. The three *Pasteuria* differed in the folding patterns of their 16S rRNA molecules, with the definitive difference occurring in the hypervariable region of helix 6.

ASSESSING SPATIAL VARIABILITY IN HETERODERA GLYCINES AS A PREREQUISITE FOR ITS SITE-SPECIFIC MANAGEMENT. **Avendaño, Felicitas,[1] F. Pierce,[2] O. Schabenberger,[3] and H. Melakeberhan.[1]** [1]Department of Entomology and [2]Department of Crop and Soil Sciences, Michigan State University, East Lansing, MI 48824-1115; [3]Department of Statistics, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061-0439.

Heterodera glycines is important because it counts for approximately 54% of yield loss in soybean in the US. The occurrence and density of *H. glycines* may vary considerably within infested fields creating the opportunity for its site-specific management. A prerequisite for site-specific management is the occurrence of moderate to strong spatial dependence in cyst incidence and population density. This study assessed the spatial variation in *H. glycines* in a Michigan field. A nested sampling design was applied, and single-core soil samples were collected at planting. Cysts were extracted from the soil and counted. The semivariogram of cyst density was fit well by an exponential model. *Heterodera glycines* showed strong spatial dependence (range = 30 m, nugget variance = 15 %). Cyst density ranged from 0 in 20% of the field to a maximum of 121 cysts 100 cm-3. Kriging was used to generate maps of cyst distribution. Clusters of cysts were present

in the field, with cyst distribution related to soil type. *Heterodera glycines* is not uniformly distributed within this field indicating that whole field management schemes would not be useful. Factors controlling cyst distribution will be explored.

POLYMORPHISMS WITHIN SYMPATRIC AND ALLOPATRIC ISOLATES OF *BELONOLAIMUS LONGICAUDATUS*. **Bekal, Sadia, I. Kaloshian, and J. O. Becker.** Department of Nematology, University of California, Riverside, CA 92521.

The sting nematode *Belonolaimus longicaudatus* parasitizes a wide host range and can cause serious economic losses on many important crops. We investigated DNA polymorphism within eleven populations of *B. longicaudatus* using ITS and RAPD analyses. The sting nematode populations used were from different geographic locations in the United States (1 each from North Carolina, Georgia and Arkansas, 3 from Florida, and 5 from California). Our preliminary results showed similarity of the restriction patterns of the ITS for all the nematode populations with TaqI and HinfI restriction enzymes. Polymorphisms for 2 Florida populations and the North Carolina population were generated using HincII and Sau3A restriction enzymes, while all other populations showed a similar restriction pattern. RAPD patterns using 12 different decamers showed a high level of variation between the different geographic populations of the sting nematode. A low level of variation was found within the California populations suggesting their gene pool similar.

PHENOTYPIC AND MOLECULAR EVIDENCE FOR A NEW *PASTEURIA* SPECIES PARASITIC ON *BELONOLAIMUS LONGICAUDATUS*. **Bekal, Sadia,[1] R. M. Giblin-Davis,[2] J. Borneman,[3] and J. O. Becker.[1]** Departments of [1]Nematology and [3]Plant Pathology, University of California, Riverside, CA 92521; [2]University of Florida, Fort Lauderdale Research and Education Center, Fort Lauderdale, FL 33314.

Strain S1 of the *Pasteuria penetrans* group was found to parasitize the sting nematode. Sporangium morphology and ultrastructure the bacterium was different from the typical *P. penetrans* species. The mature spore is approximately twice as large as *P. penetrans*. Attachment tests demonstrated its host specificity to *B. longicaudatus*. S1 spores did not adhere to *Meloidogyne incognita, M. javanica, M. hapla, Pratylenchus brachyurus, P. scribneri, P. neglectus, P. penetrans, P. thornei, P. vulnus, Xiphinema* sp., *Longidorus africanus,* and *H. schachtii*. Spores attached to several sting nematode populations from different geographical origins in the United States. Genomic DNA was isolated from a mature endospore suspension of an in-vivo culture of S1-*Pasteuria* sp. A 1.4 Kb fragment of the 16S rDNA was PCR amplified. Sequence data of the 16 S rDNA gene from the S1-*Pasteuria* sp. isolate showed 96% identity with *P. penetrans* and 93% identity with *P. ramosa*, supporting its classification as a new species.

EFFECT OF GRAIN SORGHUM, GRAIN AND FORAGE PEARL MILLET ON *PRATYLENCHUS PENETRANS* POPULATIONS ON TOBACCO IN QUEBEC. **Bélair, Guy, Y. Fournier, and N. Dauphinais.** Agriculture and Agri-Food Canada, St-Jean-sur-Richelieu, Quebec, Canada J3B 3E6.

A two-year crop rotation experiment was conducted in 1998-1999 to assess the impact of grain sorghum and both, forage and grain pearl millet on *Pratylenchus penetrans* populations in a tobacco field at St-Étienne-des-Grès, Quebec. In 1998, rotation crops were: forage millet FMH2, grain millet GMH6, grain sorghum GS7, and rye cv. Musketeer. Fumigated rye was also included as a control. In 1999, all plots were grown in tobacco.. Forage millet and, to a lesser extent, grain millet used as a rotation crop significantly reduced the number of root-lesion nematodes in both soil and tobacco roots when compared to rye, the traditional rotation crop. Grain sorghum increased root-lesion nematode populations. The suppressive effect of both millets was persistent in plots where tobacco was grown in 1999. The best yields of tobacco were recorded in plots previously grown in forage millet and were comparable to the yields obtained in the fumigated rye plots. Forage and grain pearl millet increased tobacco plant height, and improved by 1.5-fold whole plant

dry weight when compared to the non-fumigated rye. Tobacco plant height, number of leaves per plant, and plant dry weight were significantly reduced in grain sorghum plots. Forage and grain millet could be useful as rotation crops to reduce *P. penetrans* populations and increase tobacco yields.

IDENTIFICATION OF A NEW *MELOIDOGYNE* SPECIES BY RAPD-PCR AND SPECIFIC PCR PARTIAL AMPLIFICATION OF THE GENE COII. **Bendezu, Ivan F., and J. L. Starr.** Department of Plant Pathology & Microbiology, Texas A & M University, 2132 TAMUS, College Station, Texas 77483-2132.

Species-specific markers for populations of an undescribed *Meloidogyne* sp. found in Collingworth County Texas, were identified using two different PCR protocols. Using RAPD-PCR with the oligonucleotide primer Operon A-01, a DNA profile with four bands of 1500 bp, 820 bp, 870 bp and 750 bp specific for these populations was amplified. Additionally, using a partial nucleotide sequence of the gene for the cytochrome oxidase subunit II (COII) gene, two specific primers MTXF and MTXR were designed. These primers amplified a band of approximately 1200 bp that distinguishes this species from *M. arenaria, M. hapla, M. incognita* and *M. javanica*. The two techniques were efficient and very reproducible when high quality DNA was used.

EMIGRATION OF SECOND STAGE JUVENILES OF *MELOIDOGYNE ARENARIA* FROM THE RESISTANT PEANUT CULTIVAR COAN. **Bendezu, I. F., N. L. Riley, and J. L. Starr.** Department of Plant Pathology & Microbiology, Texas A & M University, 2132 TAMUS, College Station, TX 77843-2132.

Pre and post-infectional behavior of second stage juveniles (J2) of *Meloidogyne arenaria* infecting roots of two cultivars of peanut (*Arachis hypogaea*), were evaluated. The number of J2 penetrating the roots of the resistant peanut cultivar COAN was lower ($P = 0.05$) than the number penetrating the susceptible peanut cultivar Florunner. Also the number of J2 emigrating from the roots of the resistant cultivar COAN was higher ($P = 0.05$) than the number emigrating from the susceptible cultivar Florunner. It was observed that the rate of development of swollen females was lower in COAN compared to Florunner. In three experiments no evidence of a hypersensitive reaction was observed in the roots of the resistant COAN. These data are evidence for a major effect resistance, conditioned by a single dominant gene that is not due to necrotic hypersensitive mechanism.

THE IMPACT OF SUB-IRRIGATION AND TILLAGE ON NEMATODE COMMUNITY STRUCTURE. **Berney, Michael, and G. W. Bird.** Department of Entomology, Michigan State University, East Lansing, MI 48824.

Water table was maintained at 50 and 75 cm below soil surface in 2 treatments by pumping water up into drain tile. Adjacent drain only tiled treatments were the controls. Alternating 6 row bands of conventional and reduced tillage crossed all treatments, blocking for tillage. Soil samples were composed of multiple 20-cm- deep cores through the root zone of the current crop, soybeans. Sampling was conducted in June, July and August. Nematodes were extracted by Baerman funnel, counted and identified to genus, and assigned to life history categories. The sub-irrigated treatments had a higher percent of total population in bacterial-feeding nematodes and a lower percent of both plant-parasitic and plant-associated nematode genera than the adjacent drain only tiled treatments. The 50-cm-water table treatment had a significantly higher percent of the population as bacterial feeders than the 75-cm-water table treatment. Conventional tillage resulted in more genera of nematodes, and a higher percentage of both bacterial-feeding and plant-associated genera than reduced tillage. The percent of carnivorous nematodes was higher in the tilled block than the reduced tillage.

MANAGING NEMATODES AS AN INTEGRAL COMPONENT OF SUSTAINABLE SOIL MANAGEMENT PRACTICES. **Bird, G. W.** Dept. of Entomology. Michigan State University, East Lansing, MI 48824.

The presentation will begin with: 1) a conceptual model of the role of nematodes in soil organic matter dynamics, 2) review of the taxa of the Nematoda in relation to ecosystem function, and 3) overview of a concept of soil quality: ''ability to accept, hold and release nutrients and other chemical constituents; accept, hold and release water to plants, streams and groundwater; promote and sustain root growth; maintain suitable soil biotic habitat; respond to management and resist degradation (Bird, Berney and Cavigelli, 1998)''. This will be followed by a discussion of the mechanistic and ecological world views of Capra (1996) as related to sustainable development, soil quality management systems and the Nematoda. The role of nematode management will be described in relation to the strategies of soil quality maintenance and enhancement: with special reference to physical, chemical, biological and integrated practices. The concept of multi-feedback loops and overlapping functions will be used to propose that nematodes are both censors and professors of soil quality (Howard, 1940; Wiener, 1961).

PHANTASTICALLY KNOTTED FEEDING SITES: A LINK BETWEEN TRANSCRIPTION FACTORS AND PHYTOHORMONES IN GIANT CELL FORMATION. **Bird, D. McK., A. E. Greene, H. Koltai, J. E. Schaff, and J. Watkins.** Department of Plant Pathology, NCSU, Box 7616, Raleigh, NC 27695.

We have identified a large suite of genes expressed in *Meloidogyne incognita*-induced tomato giant cells and have begun to dissect the cascade of host gene expression necessary for feeding-site formation and function. We are especially interested in transcription factors that mediate between external hormonal cues and events within the developing giant cells. Because of their role in maintenance of meristems, we have focussed our attention on PHAN and KNOX; PHAN encodes a Myb transcription factor postulated to repress the KNOX homeobox gene. Sequence analysis revealed that the DB#280 clone we previously isolated from giant cells encodes the tomato orthologue of PHAN (Le-phan). Using an in situ approach, we mapped the spatial and temporal distribution of Le-phan transcripts in sections of healthy and nematode-infected tomato tissue, confirming expression in meristems and giant cells. Strikingly, we found the Le-phan profiles to be coincident with those of the Tkn2 KNOX gene, suggesting that, in tomato at least, PHAN alone is insufficient to repress KNOX. Further, these results imply that giant cells exhibit meristematic characteristics. Perhaps associated with its function in meristem maintenance, it has been proposed that ectopic KNOX expression leads to aberrant auxin transport. Interestingly, disruption of polar auxin flow at feeding sites has recently been documented, and we will establish the relationship between Tkn2 expression and auxin flow in developing galls. Further, a strict correlation between KNOX and cytokinin levels has been observed in the shoot apical meristem and we currently are mapping the spatial and temporal induction of cytokinin-responsive promoters during giant cell initiation to correlate this with Le-phan and Tkn2 expression. It is clear that phytohormone levels are altered during establishment of the parasitic interaction; understanding where in the interaction these changes exert their influence (and on what) will likely reveal much about how giant cells are formed.

USE OF A GAL4-GFP ENHANCER TRAP TO MONITOR GENE EXPRESSION IN *ARABIDOPSIS* ROOTS INFECTED WITH *MELOIDOGYNE JAVANICA*. **Blinco, J., R. H. Potter, and M. G. K. Jones.** Western Australian State Agricultural Biotechnology Centre, Murdoch University, Perth, Western Australia, 6150.

A culture system has been developed to allow confocal laser scanning microscopy observations of nematode feeding sites during the entire life cycle of roots infected by endoparasitic nematodes. Transgenic *Arabidopsis thaliana* plants with an enhancer trap based on yeast GAL4 and an upstream activator sequence (UAS) linked to a GFP marker gene have been used to monitor

changes in gene expression in and around giant cells induced by *Meloidogyne javanica*. These transgenic lines have different cell lineages tagged with GFP. In one line, in which GFP in control roots was only expressed in phloem cells, strong up-regulation of GFP was also found inside giant cells. In another line, in which the normal expression of GFP only occurred in cells of the endodermis, there was complete down-regulation of GFP in cells surrounding the giant cells. This result suggests that a functional endodermal cell layer may not be present around giant cells. This system is a novel way of studying gene expression at nematode feeding sites, in vivo, over the time course of the host-parasite interaction. The potential also exists for isolation of tagged regulatory sequences important in nematode-plant interactions.

PROSPECTS FOR MANAGING *GLOBODERA PALLIDA.* **Blok, V. C., M. S. Phillips, M. R. Armstrong, J. T. Jones, and D. L. Trudgill.** Department of Mycology, Bacteriology and Nematology, Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA, UK.

The management of *G. pallida* is severely constrained by the lack of single major gene resistance that can be introduced into commercially attractive potato cultivars. To gain a better understanding of the opportunities and limitations of the use of available quantitative resistance, both biological and molecular studies were undertaken to assess the intraspecific variation in *G. pallida*. A considerable range in reproductive capacity on resistance derived from *Solanum vernei* and *S. tuberosum* ssp. andigena CPC 2802 was observed both with field populations and those produced by artificial fragmentation and selection. Molecular studies of these populations also indicate an inherent heterogeneity suggesting that multiple host and pathogen genes are involved in the host-nematode interaction. The significance of this variation in relation to managing *G. pallida* is discussed.

VIRULENCE AND AVIRULENCE OF ROOT KNOT NEMATODES IN TOMATO. **Blok, V. C.,[1] M. R. Armstrong,[1] E. A. Tzortzakakis,[2] and M. S. Phillips.[1]** [1]Department of Mycology, Bacteriology and Nematology, Scottish Crop Research Institute, Invergowrie, Scotland DD2 5DA and [2]Plant Protection Institute, National Agricultural Research Foundation, P.O. BOX 1802, 71110, Heraklion, Crete, Greece.

The majority of nematode-infested vegetable growing areas of Crete were found to contain *Meloidogyne javanica.* Most of these populations were avirulent on tomato carrying the *Mi* gene. However, a few virulent *M. javanica* populations were also identified. In an AFLP comparison of both virulent and avirulent populations, a very high mean similarity between these populations was found, suggesting that both virulent and avirulent populations were probably derived from the same founder populations. We have been comparing expressed gene sequences of both virulent and avirulent populations and compatible and incompatible responses using cDNA AFLPs and suppressive subtractive hybridization to identify pathogenicity factors and host genes with altered expression.

CO-SPECIACION OF ENTOMOPATHOGENIC NEMATODES *(STEINERNEMATIDAE* AND *HETERORHABDITIDAE)* AND THEIR SYMBIOTIC BACTERIA. **Boemare, Noël.** Laboratoire de Pathologie comparée, UMR CNRS-INRA-UMII n° 5087, CP 101, University of Montpellier II. F-34095 Montpellier CEDEX 5, France.

Bacteria of the genera *Xenorhabdus* and *Photorhabdus* are symbiotically associated in the gut of infective juveniles of entomopathogenic nematodes, *Steinernema* and *Heterorhabditis.* A polyphasic approach, including many phenotypic tests, restriction polymorphism and sequencing analysis of PCR-amplified 16S rRNA genes, and DNA-DNA hybridizations with determination of the DTm, is required to characterize species/strains of both bacterial genera. Several studies indicate that nearly every species of entomopathogenic nematodes possess a specific symbiont species or subspecies. Up to date, there are five described *Xenorhabdus* species and it has been demonstrated that one *Xenorhabdus* species can be shared by several *Steinernema* species. As for *Heterorhabditis*

symbionts, three *Photorhabdus* species, have been described. When we compare these results with the available phylogenetic trees of their nematode hosts, a close relatedness of the two taxonomic structures is noticed, and a phenomenon of co-speciation between bacterium and nematode genera is shown. It is believed that the similarities between the two sister genera, *Xenorhabdus* and *Photorhabdus,* are the result of a convergent evolution between two different bacterial genera associated with two phylogenetically different nematode genera, *Steinernema* and *Heterorhabditis,* respectively.

MOLECULAR DIAGNOSTICS OF VIRUS VECTOR TRICHODORIDS. **Boutsika, Konstantina,[1] V. C. Blok,[1] M. S. Phillips,[1] S. A. MacFarlane,[2] and D. J. F. Brown.[1]** [1]Unit of Mycology, Bacteriology & Nematology, and [2]Unit of Virology, Scottish Crop Research Institute, Invergowrie, Dundee, DD2 5DA, Scotland,UK.

Tobacco rattle tobravirus (TRV) occurs worldwide but is particularly prevalent in Europe and North America. The virus is transmitted from plant to plant by soil-inhabiting ectoparasitic nematodes belonging to the genera *Paratrichodorus* and *Trichodorus.* Powerful new DNA-based approaches for specimen identification have been developed and successfully adapted for application in trichodorids. A PCR assay with ribosomal DNA primers derived from ITS-sequences has been developed which reliably distinguishes individual *P. anemones, P. pachydermus, T. primitivus* and *T. similis* nematodes from each other. Cloning and sequencing of the amplified products is in progress to identify species-specific primers. Concurrently, a RT-PCR assay was developed that reliably detects the presence of TRV in individual trichodorids.

IMMUNOLOCALIZATION OF ADHESINS IN *PASTEURIA* SPP**.** **Brito, J. A.,[1] J. F. Preston,[2] D. W. Dickson,[1] R. M. Giblin-Davis,[1] and H. Aldrich.[2]** [1]Entomology and Nematology Dept., and [2]Microbiology and Cell Biology Dept., University of Florida, 32611 Gainesville, FL.

Previous studies used an IgM monoclonal antibody to detect the appearance of adhesins during sporogenesis and to follow their distribution during the development of *Pasteuria penetrans* (P20 isolate). In this study, this IgM was used to detect and to localize the adhesins in two species of *Pasteuria* (soybean cyst *Pasteuria* and sting *Pasteuria* [S-1]), three undescribed species of *Pasteuria* (lance *Pasteuria* [L-1 and LS-1]), ring *Pasteuria* (C-1), and one *P. penetrans* isolate (P-1). TEM was used to exam sections of *Pasteria* infected nematodes. Nematodes were fixed, dehydrated, and embedded in LR White resin. Sections were labeled with anti-P-20 IgM and anti-mouse IgM conjugated with colloidal gold. Antigens bearing the epitope were uniformly distributed in the sporangium, and exosporium, but not in the parasporal fibers. This indicates that the epitope that was recognized by the IgM in the P-20 isolate is shared among other species of *Pasteuria*. This epitope, which was not found associated with any of the several *Bacillus* spp. previously examined is considered to be a component of a recognition system shared by different strains and species of *Pasteuria*.

MANAGEMENT OF MULTIPLE PATHOTYPES OF GLOBODERA ROSTOCHIENSIS WITH HOST RESISTANCE. **Brodie, B. B., and D. M. Thurston.** USDA, ARS, and Department of Plant Pathology, Cornell University, Ithaca, NY 14853.

Several clones with resistance to *Globodera rostochiensis* pathotype Ro2 were identified in a potato breeding population with a pedigree of *Solanum tuberosum* ssp. *tuberosum, S. tuberosum* ssp. *andigena,* and *S. vernei*. Intercrossing these clones and outcrossing their progeny to cultivars with Ro1 resistance produced several clones with resistance to both pathotypes. Two of these clones were tested in field plots with mixed infestations of pathotypes Ro1 and Ro2. Controls were cultivars Kanona that is resistant to pathotype Ro1 and Katahdin that is susceptible to both pathotypes. Experimental plots were two rows 1 m apart and 3 m long and each clone or cultivar was replicated four times. Soil samples consisting of 20 probes 6 cm deep were taken from each plot after harvest of each year. Cysts were extracted from a 300 cc subsample of soil from each plot

and crushed to determine the number of eggs. After two years, the *G. rostochiensis* population density increased 228% following the cultivar Katahdin that has no resistance and 26% following the cultivar Kanona that is resistant to only pathotype Ro1. The *G. rostochiensis* population density decreased 96% after two years of growing the advanced clones R6-4 and NY121 that possess resistance to both pathotypes.

LATENT INFECTION IN ENTOMOPATHOGENIC NEMATODES: A POSSIBLE OVERWINTERING STRATEGY. **Brown, Ian,[1] B. Lovett,[1] P. Grewal,[2] and R. Gaugler.[1]** [1]Department of Entomology, Rutgers University, New Brunswick, NJ 08901 and [2]Department of Entomology, Ohio State, Wooster OH 44691.

Under optimal temperatures, entomopathogenic nematodes release their bacteria upon host entry; killing the insect within 24 to 48 hr. We hypothesize that 1) low temperatures induce latency in the infection process, 2) that this latency provides a means for entomopathogenic nematodes to overwinter within the host and 3) On warming, the normal infection process resumes. *Galleria mellonella* were infected with 50 infective juveniles of *Steinernema carpocapsae, S. riobrave, S. scapterisci, Heterorhabditis bacteriophora* and *H. megidis* at 10°C. All species demonstrated establishment, that resulted in mortalities on warming at 25°C after 20 days at 10°C. The effect was examined in more detail using *Heterorhabditis bacteriophora* to infect *G. mellonella, Popillia japonica* and *Exomala orientalis* larvae at 25, 15, 10 and 5°C and incubated for 3, 10, 25 and 35 days These data lends support to our hypothesis on entomopathogenic nematode overwintering strategies.

CRACKING THE EGG: TARGETING GENES RESPONSIBLE FOR THE DEVLOPMENT OF *HETERODERA GLYCINES*. **Burgwyn, B., and R. I. Bolla.** Department of Biology, Saint Louis University, St. Louis, MO 63103-2010.

Over a billion dollars of the annual loss in soybean crop yield worldwide can be attributed to the plant parasitic nematode *Heterodera glycines*, the soybean cyst nematode (SCN). SCN is able to remain in the soil and can survive adverse conditions. To combat SCN from parasitizing soybean it is important to understand the molecular mechanisms that are responsible for SCN development. Genes transcribed during embryogenesis can be targeted to arrest the nematode's development and induce a permanent dormancy before the nematode is able to hatch or promote the nematode to hatch without the presence of a host. We have elucidated that SCN encodes a gene homologous to hch-1 from *Caenorhabditis elegans.* The *C. elegans* gene contains metalloprotease, CUB, and EGF-like domains. One of these domains is transcribed during early embryogenesis of SCN. Furthermore, we have determined that there are hch-1 homologous sequences in other cyst nematodes. Hch-1-like genes are likely targets to interrupt development of SCN and prevent parasitism.

THE EFFECT OF HATCHING FACTORS, PLANT GROWTH REGULATORS AND OTHER ORGANIC COMPOUNDS ON THE MOVEMENT OF SECOND STAGE JUVENILES OF *GLOBODERA ROSTOCHIENSIS*. **Byrne, John T. and B. B. Brodie.** USDA-ARS Plant Protection Research Unit, U.S. Plant, Soil and Nutrition Lab., Tower Rd., Cornell University, Ithaca NY 14853.

It has been suggested that second stage juveniles (J2s) of the potato cyst nematodes (PCN) may use chemical signals from host roots to identify appropriate invasion sites (such as behind the root tip). Preliminary studies demonstrated the ability of J2s to orientate towards the roots of its primary host plant, potato. The effect of several potential chemo-attractants on juvenile movement in sand columns was assessed. Potato root diffusate (PRD), certain G-10 separated PRD fractions and several other compounds stimulated significant movement of water-hatched J2s. These included IAA (10-10M), IBA (10-11M) and a mixture of eight different cytokinins identified in PRD. In addition, secretions from a large number of water-hatched J2s also stimulated significant J2

movement. These and other compounds were also screened for their ability to attract J2s over a concentration gradient using both agar and sand based assay systems.

MOLECULAR AND MORPHOLOGICAL CHARACTERIZATION OF THREE NEW *PRATYLENCHUS* SPECIES. **Carta, Lynn K., A. M. Skantar, and Z. A. Handoo.** Nematology Laboratory, USDA-ARS, Beltsville, MD 20705.

Morphometrics and photographs of three new *Pratylenchus* species and their proposed relationships to other species of Pratylenchinae using 28SrDNA are presented. Two new South African species from cotton are related to one another morphologically, differing in length of stylet and postvulval sac, presence or absence of lateral line crenations and tail projections, number of tail annules, shape of stylet knobs and median bulb, and behavior. Their 28SrDNA sequences differ by only 2 deletions /300 bp (base pairs) from one another, and by 16/300 bp from *Nacobbus aberrans*. The closest morphological relative is *P. teres* with 6 lateral lines. A new species from grass in Arlington Cemetery, Virginia, U.S.A. has the unusual morphological character among *Pratylenchus* species of 6-8 crenate lateral lines and a pyriform to slightly overlapping basal bulb. Molecularly, the 28SrDNA sequence of the Arlington Cemetery species is closely related to *P. convallariae* (3/300 bp) and more distantly to *P. hexincisus* (45/300 bp).

USE OF TRAP CROPS WAS NOT AN EFFECTIVE MEANS OF MANAGING SOYBEAN CYST NEMATODE. **Chen, S. Y.,[1] P. M. Porter,[2] C. D. Reese,[1] and W. C. Stienstra.[3]** [1]University of Minnesota Southern Research and Outreach Center, Waseca, MN 56093; [2]Department of Agronomy and Plant Genetics; and [3]Department of Plant Pathology, University of Minnesota, MN.

A study was conducted out at two field sites in 1998 and 1999 to evaluate the potential of trapping soybean cyst nematode (SCN) with soybean and pea in the corn year to manage SCN in Minnesota. In 1998, treatments included four seeding rates (0, 124, 247, and 494 $\times 10^3$ seeds/hectare) of resistant soybean and four killing dates (3, 4, 5, and 6 weeks after planting) of soybean with glyphosate herbicide. In 1999, the experiment included four trap crop comparisons (resistant soybean at 494 $\times 10^3$ seeds/hectare, susceptible soybean at 494 $\times 10^3$ seeds/hectare, pea at $1{,}482 \times 10^3$ seeds/hectare, and no trap crop) and five killing dates (3, 4, 5, 6 weeks after planting, and no killing). Six replicates were used. Each plot consisted of four 76-cm corn rows, 7.6 m long. Trap crops were planted before planting corn on the same day at each site. Plots were separated by four rows of corn without trap crop. Nematode egg densities were determined at planting, 1 month and 2 months after planting, and at harvest. Corn yield was recorded. No significant difference in the nematode population density was observed among the treatments at either site. Treatments did not affect corn yields. These results suggest use of soybean and pea as trapping crops during the corn year was not an effective means for SCN management.

GENETIC VARIATION WITHIN *MELOIDOGYNE HAPLA* RACE A. **Chen, Peichen, and P. A. Roberts.** Department of Nematology, University of California, Riverside, CA 92521-0415.

Seven geographically distinct isolates of *Meloidogyne hapla* were collected and maintained in the greenhouse on tomato (*Lycopersicon esculentum* cv. VFN-8). Young adult females were extracted at three different times within eight months and processed for GOT isozyme analysis. The GOT isozyme patterns revealed that all seven isolates were race A. Previous work had identified genes for resistance to root-knot nematodes in common bean (*Phaseolus vulgaris*). Resistant cultivar NemaSnap, which contains one or both of resistance genes *Me2* and *me3*, was challenged with inoculum of each of the seven *M. hapla* isolates. The tests were made in 5 replicates in growth pouches by inoculating 10-day-old seedlings with approximately 1200 J2 per plant. The numbers of egg masses produced per root system were analyzed by Duncan,s multiple range test. Three *M. hapla* isolates AN, TN, LM were found to be virulent on cv. NemaSnap, producing high numbers of egg masses similar to those produced on susceptible cv. Yolano. Isolates PX and K6 were found to be avirulent on NemaSnap, producing low egg mass numbers, while isolates SB and WI

produced few egg masses on either resistant NemaSnap or susceptible Yolano, indicating a weak host reaction. Preliminary analyses of the seven isolates by RAPD-PCR revealed that some OPA primers produced different banding patterns between these isolates.

CONSIDERATION OF THE INFLUENCE OF NEMATOPHAGOUS ORGANISMS AND RECYCLING THROUGH INSECT HOSTS ON THE SURVIVAL OF ENTOMOPATHOGENIC NEMATODES IN THE FIELD. **Chevalier, R., and J. M. Webster.** Department of Biological Sciences, Simon Fraser University, 8888 University Drive, Burnaby, B.C., Canada, V5A 1S6.

Laboratory studies have shown that entomopathogenic nematodes (EPNs) have a wide host range among soil-dwelling insects, in addition to themselves being prey to soil organisms such as mites, collembolans, nematophagous fungi and predaceous nematodes. The presence of EPN antagonists and of the target and several non-target hosts could explain the variability in EPN survival under field conditions. In order to study these dynamic biotic interactions influencing the survival and subsequent efficacy of EPNs, a study is in progress to determine the impact of *Heterorhabditis marelatus* inundative field release on the population dynamics of the main nematophagous soil organisms and on target and non-target insects in a grassland ecosystem in British-Columbia.

BIOLOGICAL CONTROL OF MELOIDOGYNE ARENARIA BY PASTEURIA PENETRANS. **Cho, Myoung Rae, Dong Soon Kim, Heung Yong, Jeon, and Myoung Soon Yiem.** Horticultural Environment Division, National Horticultural Research Institute, Suwon. 441-440, Korea.

Pasteuria penetrans 98-35(PP), isolated from oriental melongreenhouse soil in Korea, was evaluated for suppression of root-knot nematode, Meloidogyne arenaria (MA), on tomato and oriental melon. Pot experiments were conducted by planting seedlings in medium inoculated with 5,000 MA juveniles(J2)/pot, J2+100,000 PP endospores/1cm^3 medium, and J2+200,000 PP endospores/cm^3 medium. After 10 weeks, root gall percentages in J2+100,000 PP endospores/1cm^3 medium and J2+200,000 PP endospores/1 cc medium were significantly lower with 37.5% and 6.7%, respectively, compared to the J2 of 85%. In the second planting of tomatoes in the same pots, root gall numbers were significantly lower in PP treated pots with 68.8 and 31.4/root in J2+100,000 PP endospores/1cm^3 medium and J2+200,000 PP endospores/1cm^3 medium, respectively, compared to the J2 of 460.6/plant. In oriental melon experiment, number of root galls after 10 weeks was significantly lower in J2+200,000 PP endospores/1cc medium with 32.5 compared to 64.1 and 87.5 in J2+100,000 PP endospores/1cc medium and the control, respectively.

PROPAGATION OF THE SOYBEAN CYST NEMATODE ON HAIRY ROOTS AND EXPRESSION OF RESISTANCE IN TRANSGENIC ROOTS. **Cho, H. J.,[1] G. R. Noel,[2] S. K. Farrand,[1] T. Eggett,[1] and J. M. Widholm.[1]** [1]Department of Crop Sciences, and [2]USDA-ARS, University of Illinois, Urbana, IL 61801.

Hairy roots of soybean were induced by *Agrobacterium rhizogenes* on *Heterodera glycines*-susceptible Lee 68, Mandarin, Maple Arrow and Williams 82, and *H. glycines*-resistant Cartter, Fayette, Hartwig, Jack, Peking and PI 437654 at rates of 54 to 95 % on a selective medium containing 200 mg/ml kanamycin and 500 mg/ml carbenicillin. Eight weeks after inoculation with *H. glycines* eggs, the number of cysts formed on hairy roots of the resistant cultivars was 0 to 2, whereas the number on the susceptible cultivars was 0 to 175, indicating that the resistance phenotype was preserved in transgenic hairy roots. Several candidate genes were introduced into Williams 82 for evaluation of SCN-resistance imparted by these genes. The expression of transgenes was confirmed by polymerase chain reaction (PCR), Southern, Northern, and Western blots. The resistance of these candidate genes to *H. glycines* is now being tested.

MORPHOLOGICAL AND MOLECULAR IDENTIFICATION OF GLOBODERA HYPOLYSI OGAWA, OHSHIMA & ICHINOHE, 1983. **Choi, Young-Eoun,[1] Kyung-Soon Park,[2] and S. E. Kim.[1]** [1]Department of Agricultural Biology, College of Agriculture Kyungpook National University, Taegu 702-701, Korea, and [2]Insect Research Division, National Plant Quarantine Service, Anyang, Korea 430-016.

A *Globodera* species was found on mugwort (Artemisia princeps Pampan) at Taegu, Korea. The species resembled *Globodera hypolysi* Ogawa, Ohshima & Ichinohe, 1983. The Korean specimens correspondedwell with the original description, except stylet length of the juveniles was slightly shorter (21 to 24 vs. 24 to 20 μm) and shorter distance from the anus to the fenestra in cyst (22 to 50 vs. 25~80 μm). There were two types of cyst shapes: a rounded type (L/W = 0.9 to 1.3) and a semi-rounded type (L/W = 1.2~1.6). The morphological characteristics revealed that these two types belong to the identical species, *G. hypolysi.* The internal transcribed spacer region (ITS1) of the nuclear ribosomal genes was amplified from individuals of both types of cysts. Amplified product size was approximately 700bp and ITS1 DNA sequences were identical to each other. The DNA sequence data were compared with four species of *G. rostochiensis, G. pallida, G. tabacum virginiae, G. tabacum solanaearum.* Phylogenic analysis of the rDNA sequence data indicated that *G. hypolysi* is relatively dissimilar to the other four species.

VIRULENCE AND PERSISTANCE OF *STEINERNEMA FELTIAE* TOWARDS HOUSE FLY, *MUSCA DOMESTICA*, IN BOVINE MANURE. **Clark, Brian P.,[1] D. B. Taylor,[2] A. L. Szalanski,[3] and T. O. Powers.[3]** [1]Department of Entomology, University of Nebraska-Lincoln, Lincoln, Nebraska 68583; [2]USDA-ARS, Midwest Livestock Insects Research Unit, Lincoln, Nebraska 68583; and [3]Department of Plant Pathology, University of Nebraska-Lincoln, Lincoln, Nebraska 68583.

The virulence of *Steinernema feltiae* strain SN towards house fly, *Musca domestica*, and nematode persistence in the bovine manure environment was examined. Nematodes were applied at the 95% LD rate of 1x106 nematodes per m2 to thirty two one liter containers containing 150 g of manure. Another thirty two containers without nematodes was the control. At weekly intervals, 100 third instar *M. domestica* larvae were added to four of the treated and four of the control containers. Survival was determined by adult house fly emergence. There was a twenty percent weekly decline in the infectivity of the nematodes towards house fly larvae. After four weeks there was no significant difference between treatment and control.

THE EFFECTS OF SOIL MOISTURE AND SOIL TYPE ON THE SURVIVAL AND PATHOGENICITY OF *HETERORHABDITIS MARELATUS.* **Cottrell, Nathan, E. Grafius, and H. Melakeberhan.** Department of Entomology, Michigan State University, East Lansing, MI 48824.

Colorado potato beetle, *Leptinotarsa decemlineata*, is among the most significant pests of potatoes, largely because it has developed resistance to many insecticides and has no effective biological control agents. The objective of this study was to determine how edaphic factors effect the survival and pathogenicity of the entomopathogenic nematode, *Heterorhabditis marelatus*. The information obtained will lead to studies on the efficacy of *H. marelatus* to control Colorado potato beetles. Two separate experiments test the effects of soil moisture and soil type on the survival and pathogenicity of *H. marelatus*. In both studies 2,000 infective juveniles were placed in 100 cc of sterile soil for four weeks. At four weeks, infective juveniles were extracted, counted, and living nematodes were separated. *Galleria mellonella* larvae were then exposed to the living portion of *H. marelatus* to compare relative pathogenicity. Dry soil and heavy soils reduced both survival and pathogenicity of nematodes.

NEMATICIDE INJECTION THROUGH DRIP TAPE IN VEGETABLE PRODUCTION. **Csinos, A. S.,[1] A. W. Johnson,[2] D. R. Sumner,[1] and T. M. Webster.[2]** [1]Department of Plant Pathology, and [2]USDA/ARS, University of Georgia, Tifton, GA 31793.

A trial evaluated fumigants and EC fumigant formulations in black plastic mulch in yellow squash double-cropped with a second crop of cucumber. Primary treatments on the first crop were: 1,3-dichloropropene plus 35% chloropicrin (1,2-D+C-35) at 178 l/ha applied by chisel injection; 1,3-D+C-35 EC at 112 l/ha injected through drip tape; 1,3-D chisel injected at 112 l/ha + chloropicrin (C) at 62 l/ha and metam sodium (MS) at 467 l/ha injected under plastic (1,2-D+C+MS); methyl bromide (MB) at 448 kg/ha chisel injected; and nontreated. On the second crop, each of the primary treatments were split into 4 treatments applied through drip tape. Treatments were: 1,3-D EC at 84 l/ha; 1,3-D+C-35 EC at 112 l/ha; MS at 476 l/ha and a nontreated control. Weight and number of squash fruit and vigor ratings were higher and root-knot indices lower for all treatments compared to the control. One, 3-D+C-35 EC-treated plots had a higher root-knot index than the other treatments. Nutsedge was not controlled well by any of the treatments. *Pythium* spp. populations were not different but populations of other fungi differed among treatments. In the second crop, nematode and weed populations and parasitic fungal populations were low and variable with little difference among plots.

SURVEY OF NEMATODES ASSOCIATED WITH CORN IN GEORGIA. **Davis, R. F.,[1] and P. Timper.[2]** [1]Department of Plant Pathology, University of Georgia, Athens, GA, 30602, and [2]Crop Protection and Management Research Unit, USDA-ARS, Tifton, GA 31973.

A nematode survey of 102 corn fields was conducted in Aug 1998 and in July and August 1999, in 11 counties in Georgia. Fifteen soil cores (2.5 cm ×15-20- cm deep) were collected from 1 ha sections of corn fields; all fields had been planted in cotton or peanut the year prior to sampling. *Meloidogyne* spp. juveniles extracted from corn field soil were transferred to tomato, allowed to mature, and identified to species. Nematodes detected from soil samples, in descending order of incidence, included *Pratylenchus* sp. (74%), *Mesocriconema* sp. (58%), *Helicotylenchus* sp. (43%), *Paratrichodorus* sp. (31%), Meloidogyne incognita (30%), Tylenchorhynchus sp. (3%), M. javanica (2%), *M. arenaria* (2%), and *Rotylenchulus reniformis* (2%). In this survey, 94% of the *Meloidogyne* spp. extracted from soil samples were *M. incognita.* A mist chamber was used in 1999 to extract nematodes from corn root fragments collected on sieves when processing the survey's soil samples. Nematodes also were extracted from three intact corn root systems per field. *Pratylenchus* spp. were recovered from 89% of the root samples collected during extraction from soil; *Pratylenchus* spp. incidence was 100% of entire corn root systems sampled. Soil samples underestimate both incidence and population levels of *Pratylenchus* sp. in corn in Georgia.

DIFFERENCES IN THE CODON USAGE OF DIFFERENT PARASITIC NEMATODE TAXA. **De Giorgi, C.,[1] F. De Luca,[2] P. Veronico,[1] M. R. Cortese,[1] and F. Lamberti.[2]** [1]Dipartimento di Biochimica e Biologia Molecolare University of Bari, Bari, Italy, and [2]Istituto di Nematologia Agraria (CNR) Bari, Italy.

The codon-choice patterns of different parasitic nematode taxa have been analyzed by using a recently developed computer program on different protein coding sequences retrieved from databases. It is shown that while in the case of plant parasitic nematodes, codon usage can be an additional tool for measuring genetic distances, in the case of animal parasitic nematodes the codon choice patterns do not follow taxonomic relationships. It is also suggested that the atypical codon usage of mitochondrial genes in animal parasitic nematodes reflects the co-evolution of mitochondrial and nuclear genomes. When a much larger sequence data set, especially from plant parasitic nematodes, will be available, a model for the evolution of selective pressure on the codon usage of parasitic nematodes can be derived.

DISTRIBUTION, SURVIVAL, AND BIOLOGICAL CONTROL POTENTIAL OF THE SOIL-BORNE BACTERIUM, *PASTEURIA*. **Dickson, D. W.** Entomology and Nematology Department, University of Florida, Gainesville, FL 32611-0620.

With the loss of many of the most efficient soil fumigants, there is a great need to develop new alternative methods of plant-parasitic nematode management. Our objective is to understand the distribution, biology, and role in soil suppressiveness of *Pasteuria penetrans* to nematodes. We find the nematode antagonist widely distributed in sandy soils throughout the state. The sporogenesis process of the bacterium has been classified into seven stages. The greatest rate of attachment and development occurs at temperatures of 30 to 35 °C. Attachment of endospores to nematodes involves glycoproteins bearing 1,4-linked N-acetyl glucosamine residues on the surface of the spores, which are recognized by lectins on the cuticle of the nematode. Previous studies using an IgM monoclonal directed against selected *P. penetrans* showed that the synthesis of adhesins in P-20 isolate was first observed in the third stage of sporogenesis. Natural soil suppressiveness against root-knot nematodes caused by *P. penetrans* has been demonstrated in several fields. Induction of suppressiveness to root-knot nematodes is attained at 100,000 endospores/g of soil. Soils inoculated with lesser amounts of endopores will become suppressive in 3 years. The maximum endospore density in soil resulting from natural amplification is estimated at 130,000 endospores/g of soil.

EFFECTS OF TILLAGE AND ROW SPACING ON SOYBEAN YIELD AND SOYBEAN CYST NEMATODE REPRODUCTION. **Donald, P. A.,[1] G. R. Noel,[2] H. Melakeberhan,[3] R. Riggs,[4] N. Atibalentja,[5] J. Faghihi,[6] J. Ferris,[6] G. Tylka,[7] D. Hershman,[8] S. Chen,[9] T. Niblack,[1] T. Anderson,[10] T. Welacky,[10] C. Grau,[11] and A. MacGuidwin.[11]** [1]Department of Plant Microbiology and Pathology, University of Missouri, Columbia, MO 65211, [2]USDA/ARS Urbana, IL 61801, [3]Department of Entomology, Michigan State University, East Lansing, MI 48824, [4]Department of Plant Pathology, University of Arkansas, Fayetteville, AR 72701, [5]Department of Crop Science, University of Illinois, Urbana, IL 61801, [6]Department of Entomology, Purdue University, West Lafayette, IN 47907, [7]Department of Plant Pathology, Iowa State University, Ames, IA 50011, [8]Department of Plant Pathology, University of Kentucky, Princeton, KY 42445, [9]Department of Plant Pathology, University of Minnesota, Waseca, MN 56093 [10]Agriculture and Agri-Food Canada, Harrow, Ontario, NOR 1G0, and [11]Department of Plant Pathology, University of Wisconsin, Madison, WI 53706.

The effects of cultural practices such as tillage, row spacing, rotation, and rotation of nematode resistant soybean germplasm have not been examined to determine their effects and interactions on soybean cyst nematode, (SCN, *Heterodera glycines*), population dynamics and soybean yield. These practices were studied at nine locations throughout the Midwest and one Canadian province. SCN-resistant soybean cultivars yielded on average 672 kg/ha more than susceptible cultivars over the course of the study. SCN reproduction was three times greater on susceptible than on resistant cultivars. In general, soybean yields were similar in no-till and conventional tillage, but the results varied by year and location. Reproduction of the nematode was 25% greater in no-till than in conventional tillage. Soybean yield was 67 kg/ha greater in narrow rows compared to medium rows and 269 kg/ha greater in medium rows than wide rows, but yield also varied by location and year. Macronutrient levels in plant tissue and soil showed no consistent change with tillage, row spacing, or soybean genotype treatment; however there was a differential uptake of calcium and magnesium between resistant and susceptible cultivars at some locations.

TYLENCHULUS SEMIPENETRANS SUPPRESSES INFECTION OF CITRUS FIBROUS ROOTS BY THE FUNGUS, *PHYTOPHTHORA NICOTIANAE* (=*PARASITICA*). **El-Borai, K. F., L. W. Duncan, and J. H. Graham.** University of Florida, Citrus Research and Education Center, Lake Alfred, FL 33850.

The most commonly encountered association between nematodes and fungi in citrus worldwide occurs between the citrus nematode *Tylenchulus semipenetrans* and the root-rot fungus *Phytophthora nicotianae* (=*parasitica*). Both organisms parasitize the cortex of the fibrous root system. The interaction between *T. semipenetrans* and *P. nicotianae* in citrus roots was evaluated in the

laboratory. Forty five-day-old *Citrus aurantium* (sour orange) seedlings growing in soil in glass test tubes were infested with 2 inoculum levels (8,000 and 80,000) of a mixture of *T. semipenetrans* eggs and second stage juveniles. After nematode infection was well established, two levels of fungal zoospores (6,300 and 63,000) were added either alone or in combination with the nematode. Both inoculum levels of the fungus alone reduced the growth of citrus seedlings compared to the nematode alone or in combination with the fungus. Fungal protein in roots infected by both organisms was 57% lower ($P = 0.001$) than when infected by only the fungus. Compared to plants infected only by *P. nicotianae*, shoot weights were 44% ($P = 0.001$) greater and root weights were 17% greater ($P = 0.05$) in plants infected by both parasites. Results of this and several other experiments reveal an antagonistic relationship between *T. semipenetrans* and *P. nicotianae*.

BONA FIDE PATHOGENS OF NEMATODES MAY HOLD GREATEST BIOCONTROL CAPABILITIES. **Esnard, Joseph.** Department of Plant Pathology, Cornell University, Ithaca, NY 14853.

Several microorganisms have been investigated and considered effective for biologically-based control of plant parasitic nematodes. A review of these organisms revealed that for a single-species application, pathogens of nematodes might hold greater promise than the non-pathogens. Non-pathogens seem to be most effective in suppressing nematode populations when applications include an organic amendment. The symposium question, ''Will any one organism control any one nematode?'' is answered in the affirmative, but with examples to show that, in general, one or more organisms with or without an organic amendment may be required for effective and sustainable biomanagement of plant-parasitic nematodes. A critical examination is made of the promise, potential, and exploitation of each of the common experimental organisms (i.e., *Bacillus, Streptomyces, Pseudomonas, Burkholderia, Pasteuria, Hirsutella, Monacrosporium, Paecilomyces, Arthrobotrys, Nectria, Verticillium* and *Nematophthora* species) being studied for effective suppression of plant-parasitic nematodes.

A NEW METHOD FOR CRUSHING CYST NEMATODES TO RECOVER EGGS. **Faghihi, J., and J. M. Ferris.** Department of Entomology, Purdue University, West Lafayette, IN, 47907-1158.

The assembly consists of a plunger, a crushing chamber and two nested screens. The plunger is a #6 rubber stopper (32 ×26 ×25 mm) and a 130 mm long metal rod attached to an electric motor. The crushing chamber is constructed with two pieces of PVC pipes, 650 and 20 mm long, both with 31 mm inside diameter, and a piece of 60-mesh screen placed between them. Rubbing the rubber stopper against the screen crushes the cysts placed in the crushing chamber. Released eggs are washed down into100 and 400-mesh screens nested together. In our hands, about 98% of cysts were crushed using this method. We observed an increase of 43% in the number of recovered eggs. Viability of the eggs remained intact, and no significant differences were found in inoculation experiments between inoculum prepared with this method and the household bleach method we have used previously.

USE OF *STEINERNEMA* SPP. AND *HETERORHABDITIS INDICA* TO CONTROL *MELOIDOGYNE JAVANICA* IN TOMATO AND SOYBEAN. **Fallon, D. J.,[1] H. K. Kaya,[2] R. Gaugler,[3] and B. S. Sipes.[1]** [1]Department of Plant Pathology, University of Hawaii, Honolulu, HI 96822, [2]Department of Nematology, University of California, Davis, CA 95616, and [3]Department of Entomology, Rutgers University, New Brunswick, NJ 08903.

Indigenous Hawaiian entomopathogenic nematodes (EPNs) *S. feltiae* and *H. indica*, a Californian isolate of *S. feltiae*, and a Texan isolate of *S. riobrave* were tested for their efficacy against *M. javanica* in the laboratory and greenhouse. A single application of 1×10^4 *S. feltiae* IJs to 100 cm^3 of sterile soil containing 500 *M. javanica* J2s (applied at the same time as *S. feltiae*) reduced the number of root-knot nematodes penetrating soybean roots after 5 days ($P < 0.05$). A single application of 1×10^4 *S. feltiae, H. indica* or *S. riobrave* applied to 450 cm^3 of sterile soil reduced

the number of *M. javanica* eggs produced after 30 days in soybean and tomato plants ($P < 0.05$). Tomatoes inoculated with 1×10^4 *S. riobrave* at time of *M. javanica* application and again 30 days later produced fewer *M. javanica* eggs after 60 days than plants that received a single application of 2×10^4 *S. riobrave*, or water only at time of *M. javanica* application ($P < 0.05$). Entomopathogenic nematodes did not adversely affect the growth or development of plants on which they were applied. *M. javanica* infested tomatoes that had received *S. riobrave* were found to have longer roots ($P < 0.05$), but lower root weights ($P < 0.05$) than plants receiving water alone. Our results demonstrate the potential use of entomopathogenic nematodes as antagonists to root-knot nematodes.

VAM FUNGI SUPPRESS *PRATYLENCHUS PENETRANS* AND INCREASE NUTRIENT UPTAKE OF APPLE ROOTSTOCK. **Forge, Thomas, A. Muehlchen, C. Hackenberg, G. Neilsen, and T. Vrain.** Agriculture and Agri-Food Canada, Pacific Agri-Food Research Center, Summerland BC Canada V0H 1Z0.

Two species of Vesicular-Arbuscular Mycorrhizal (VAM) fungi, *Glomus intraradices* and *Glomus mosseae*, were evaluated in a 2-year field experiment and greenhouse experiments for their effects on growth and nutrient uptake of Ottawa 3 apple rootstock in soil infested with *Pratylenchus penetrans*. Plants were inoculated with one of the two species of VAM fungi prior to planting into fumigated and non-fumigated field plots. Non-fumigated field plots were naturally infested with the nematode and fumigated plots were re-infested with the nematode at the time of transplanting. After two seasons, the abundance of vesicles and arbuscules was greater in roots of plants inoculated with VAM fungi than in roots of non-inoculated plants, in both fumigated and in non-fumigated plots. Inoculation with VAM fungi increased plant growth and leaf concentration of P, Cu, and Zn in fumigated plots, but not in non-fumigated plots. Inoculation with VAM fungi reduced populations of *P. penetrans* in roots and soil, and the effect was most pronounced in fumigated soil. In greenhouse studies, colonization of apple roots by VAM fungi was not affected by nematode inoculation. *G. mosseae* increased total dry weights of rootstock plants in both nematode-infested and non-infested soil. Rootstocks inoculated with *G. mosseae* and *G. intraradices* supported significantly fewer *P. penetrans* per g root than rootstocks with *G. etunicatum* and *G. clarum*, but were not different from non-inoculated controls.

ENTOMOPATHOGENIC NEMATODES AND SYMBIOTIC BACTERIA INTERACTIONS. **Forst, S., A. Volgyi, and B. Boylan**. Department of Biological Sciences, University of Wisconsin, Milwaukee, WI 53201.

Xenorhabdus nematophilus is an insect pathogen that lives in a symbiotic association with the entomopathogenic nematode, *Steinernema carpocapsae.* The bacteria, which inhabit an intestinal sac of the infective juvenile stage of the nematode, are carried into the susceptible insect host and are subsequently released into the hemolymph where they participate in killing the insect. We study bacterial genetic systems that are involved in the interactions between the bacterium and the nematode. Variant cells that arise spontaneously during prolonged culturing of *X. nematophilus* are altered in numerous phenotypic properties, and are defective in their ability to interact with the nematode. In contrast, the variant strains are as virulent as the wild-type strains. To understand the genetic mechanisms involved in variant cell formation, and in the interaction with the nematode, a transposon mutagenesis approach was taken. The phenotype of the mutant strain, ANV2, mimicked that of the variant strains. A novel gene, var1, was shown to be inactivated in ANV2. While the nematode grew and developed normally on ANV2, its emergence from the nematode was delayed relative to the emergence from the wild-type strain. Transfer of var1 into ANV2 restored the phenotypic traits to the wild type state. The complemented ANV2 also emerged from the nematode at the same time as the wild-type strain. These results indicated that inactivation of a single gene was sufficient to promote variant cell formation and affected the interaction between

the bacterium and the nematode. We also study the role of the global regulatory protein, OmpR, in bacterial-nematode interactions. The ompR-minus strain was defective in the production of outer membrane proteins OpnP and OpnB, demonstrated hyperswarming activity and hyperproduction of hemolysins. This mutant strain was fully virulent towards insect hosts. However, when nematodes were grown on a mixture of the wild-type and ompR-minus strains, only the wild-type strain was recovered. The global regulatory function of ompR in *X. nematophilus* is currently being studied.

DISCOVERY OF *PHASMARHABDITIS HERMAPHRODITA* IN CHILE AND ITS PATHOLOGICAL DIFFERENCES WITH THE UK ISOLATE IN SLUG CONTROL. **France, A., and M. Gerding.** INIA–Quilamapu, P.O. Box 426, Chillán, Chile.

Biological control of slugs has been a complicated task due to the presence of few natural enemies. However, a parasitic nematode (*Phasmarabditis hermaphrodita*) was found in Chile, following a fortuitous slug sampling in 1996. This species was compared with the one discovered in the UK, during 1988. Both isolates behaved similarly, producing a swelling of the mantle, inducing feeding inhibition and body disintegration. Nevertheless, only the Chilean one produces mouth and reproductive system prolapsing. Major differences were in their symbiotic bacteria. Originally, the UK nematode has *Pseudomonas fluorescens*, as the most effective bacteria in killing slugs, while another species support nematode reproduction (*Moraxella osloensis*). The Chilean one has two associated bacteria (*P. fluorescens and Serratia fonticola*), both of them are capable to kill slugs by themselves and support nematode reproduction. In microplot trial, the commercial dose of nematodes (300.000/m2) discriminated after 4 days between both isolates ($P<0.05$) when they reached 72 and 40% of slug mortality with the Chilean and UK isolate, respectively.

DOES ACID RAIN ACCELERATE OR SUPPRESS THE PINE WILT DISEASE DEVELOPMENT? **Futai, K., and E. Asai.** Laboratory of Environmental Mycoscience, Graduate School of Agriculture, Kyoto University, Kyoto, Japan.

Current year seedlings of Japanese black pine, *Pinus thunbergii*, were pretreated with three kinds of simulated acid rain, or with distilled water for two months, then received pathogenic isolates of *Bursaphelenchus xylophilus*. Inoculum densities (Pi) were adjusted to 50, 160, or 500 for each seedling. From the day seven and on, mortality ratio of the inoculated seedlings was examined every third day. The values of mortality ratio obtained at each time were plotted against the nematode infection ($=$ Log (Pi) $\times$ days elapsed), and fitted to logistic curve. Resulting curves were compared between treatments. The slope and x-intercept of the curves represent the rate of symptom development, and the mortality zero point, respectively. The nematode inoculum loard needed to initiate seedling death was highest in the pH3 treatment. The rate of symptom development was highest in the seedlings exposed to pH 2 solution than those exposed to pH 3 or distilled water. This result suggests that pH 2 acid rain accelerates the pine wilt disease.

OBSERVATIONS ON PARASITIZED EGGS FROM A BEET CYST NEMATODE-SUPPRESSIVE FIELD. **Gao, X., and J. O. Becker.** Department of Nematology, University of California, Riverside, CA 92521-0415.

At a field location near the University of California–Riverside, a population of *Heterodera schachtii* did not increase despite continuous cropping to susceptible hosts and suitable abiotic conditions. Cysts were recovered from the field and the eggs within were observed under high power magnification. Eggs were often filled with fungal mycelium. Parasitized eggs were sometimes stained red to brown, suggesting the presence of metabolic compounds produced by the fungi. The most frequently isolated fungi from parasitized nematode eggs belonged to the genus *Fusarium.*

PLANT GENE EXPRESSION IN NEMATODE FEEDING SITES. **Gheysen, Godelieve,[1,2] K. Karimi,[1] I. Vercauteren,[1] and M. Van Montagu.[1]** [1]Laboratorium voor Genetica, Departement Genetica, Vlaams Interuniversitair Instituut voor Biotechnologie (VIB), Universiteit Gent, K.L. Ledeganckstraat 35, B-9000 Gent, Belgium; and [2]Vakgroep Moleculaire Biotechnologie, Faculteit Landbouwkundige en Toegepaste Biologische Wetenschappen, Coupure Links 653, B-9000 Gent, Belgium.

The development of nematode feeding sites is governed by differential regulation of many plant genes. Approaches to identify these genes involve comparisons between infected and control roots, including protein analysis, screening of cDNA libraries, differential display of transcribed sequences and the analysis of reporter genes in transgenic plants, either fused to known promoters or in a promoter-trap strategy. Random *in vivo gus* fusions have particularly been successful in identifying plant promoter sequences that are highly activated in nematode feeding sites, with very little expression elsewhere in the plant. These promoters are activated in giant cells as well as syncytia, and even in galls induced by the ectoparasitic nematode *Xiphinema*. Although T-DNA tagging has thus been particularly successful in identifying plant promoter sequences that are highly activated in nematode feeding sites, the isolation of the corresponding genes is usually not that straightforward. Nevertheless, the term ''cryptic promoter'' should not be used too easily, because the T-DNA is not necessarily located inside or downstream of the ''tagged'' gene. In the ARM1 line the available evidence points to the location of the tagged gene upstream (in opposite orientation) of the T-DNA. A faster way to directly identify ''real genes'' that are upregulated in the early steps of the infection process is the differential display technique. By comparing control and nematode infected *A. thaliana* roots at several timepoints in the first week after inoculation, 75 putatively upregulated sequences have been identified. Six of those have been studied in detail using *in situ* hybridisations and this analysis revealed that the upregulation is not always (only) in the feeding sites but very often (also) in the surrounding tissues such as the endodermis of the gall.

THE *FERGUSOBIA/FERGUSONINA* GALL-FORMING COMPLEX FOR BIOCONTROL OF *MELALEUCA QUINQUENERVIA* IN FLORIDA. **Giblin-Davis, R., B. Center, J. Makinson, M. Purcell, S. Scheffer, W. K. Thomas, K. Davies, G. Taylor, K. Morris, J. Goolsby, and T. Center.** University of Florida, 3205 College Avenue, Ft. Lauderdale, FL 33314-7799.

Fergusobia nematodes and *Fergusonina* flies are mutualists that cause galls on myrtaceous plant buds and young leaves. Members of the gall complex were collected from 29 different host species from North Queensland to South Australia. Sequence comparisons within flies (mtDNA) and nematodes (rRNA) showed a high degree of host specificity. Histological sections showed that galled cells were similar between different gall types and comparable to cells associated with galling by nematodes in the Anguinidae. On *Melaleuca quinquenervia*, which is an invasive weed in the Florida Everglades, the gall complex was studied in detail for possible use as an introductive biocontrol agent. Flies create diagnostic oviposition scars that can be used for no choice oviposition tests for host specificity screening. Juvenile nematodes and fly eggs were deposited into apical regions of developing buds where nematodes appeared to induce hypertrophied, uninucleate cells prior to hatch of fly eggs.

COMPARISON OF COLLAGEN GENE STRUCTURE AND EXPRESSION IN SUB-FAMILIES FROM *GLOBODERA PALLIDA* AND *CAENORHABDITIS ELEGANS.* **Gray, Lindsey Jane,[1] I. L. Johnstone,[2] V. C. Blok,[1] and J. T. Jones.[1]** [1]Unit of Mycology, Bacteriology and Nematology, Scottish Crop Research Institute, Invergowrie, Dundee, UK, and [2]Welcome Unit of Molecular Parasitology, University of Glasgow, Glasgow, UK.

We have isolated two full-length genomic sequences from the potato cyst nematode *Globodera pallida*, which encode the collagen proteins Gp-col-1 and Gp-col-2. A third, truncated collagen gene was also isolated which appears to represent an unexpressed pseudogene. All three sequences show high similarity to each other and to the previously isolated *G. pallida* cDNA clone gp-col-8.

The number and spacing of certain conserved cysteine residues as well as sequence similarity in the non (Gly-X-Y) repeat regions in these genes suggests that these molecules are all members of the same subfamily of collagen genes. Southern blotting data indicates that this family is composed of between six and ten genes. Comparison of these sequences with collagen genes from *C. elegans* suggests that the *G. pallida* sequences are most closely related to the group 1a collagens of this species. This group contains twelve genes which are not defined mutationally and remain to be functionally characterised. We present data on the expression patterns of collagen genes belonging to this subfamily from both species.

INTRASPECIFIC POLYMORPHISM IN *HETERODERA SCHACHTII* INFERRED FROM DALP ANALYSIS. **Grenier, E.,[1] A. Leroux,[1] G. Caubel,[1] C. Porte,[1] and J. Müller.[2]** [1]UMR INRA/ENSAR Biologie des Organismes et des Populations appliquée à la Protection des Plantes, 35653 Le Rheu, France, and [2]BBA Institute for Nematology and Vertebrate Research, D-48161 Münster, Germany.

Direct Amplification of Length Polymorphisms (DALP) was used to characterize eight field populations and two artificial populations of *H. schachtii* selected in laboratory conditions againt the Hs1pro1 resistance gene. Selected populations were in fact obtained from the same field population and against the same gene but in two laboratories using different plants. Intraspecific variability in *H. schachtii* was evaluated using populations from different geographic areas. No correlation was observed between the molecular clustering obtained and the geographic origins of the populations involved in this study. Selection for virulence against Hs1pro1 results in modifications of the fingerprinting pattern of the selected populations compared to the original population. These observed differential bands can now be easily sequenced as DALP markers already contain the core sequence of the universal M13 sequencing primers.

VARIATION IN VIRULENCE OF HETERORHABDITID NEMATODES AGAINST WHITE GRUBS (COLEOPTERA: SCARABAEIDAE). **Grewal, P. S., Y. Zhang, X. Wang, S. K. Grewal, and V. S. Malik.** Department of Entomology, Ohio State University, OARDC, Wooster, OH 44691-4096.

Virulence of entomopathogenic nematodes, *Heterorhabditis bacteriophora* HP88 strain, *H. bacteriophora* GPS11 strain, *H. indica*, *H. marelatus*, *H. megidis*, and *H. zealandica* against four white grub species was evaluated in sand-based bioassays. The nematode species/strains were ranked based on the LD50 (lethal dose to 50% mortality) from lowest (most virulent) to the highest against each grub species. Against the Japanese beetle, *Popillia japonica*, the nematode species ranked as follows: *H. zealandica* <*H. bacteriophora* GPS11 <*H. megidis* <*H. marelatus* <*H. bacteriophora* HP88 <*H. indica*. Against the northern masked chafer, *Cyclocephala borealis*, the species ranked as *H. zealandica* <*H. megidis* <*H. bacteriophora* GPS11 <*H. bacteriophora* HP88. Against the oriental beetle, *Exomala orientalis*, the rankings were: *H. bacteriophora* GPS11 <*H. zealandica* <*H. megidis* <*H. bacteriophora* HP88. The European chafer, *Rhizotrogus majalis* was least susceptible to entomopathogenic nematodes with *H. megidis* and *H. zealandica* producing only 25 and 15% mortality, respectively. The reasons for differences in the virulence of nematode strains to different grub species are being explored.

FIRST NORTH AMERICAN SURVEY FOR THE RECOVERY OF NEMATODES ASSOCIATED WITH MOLLUSKS. **Grewal, S. K.,[1] P. S. Grewal,[1] I. Brown,[2] L. Tan,[1] R. B. Hammond,[1] and R. Gaugler.[2]** [1]Department of Entomology, Ohio State University, OARDC, Wooster, OH 44691-4096, and [2]Department of Entomology, Rutgers University, New Brunswick, NJ 08901.

A survey was conducted to recover nematodes associated with mollusks (slugs and snails) in North America during 1999–2000. Over 10,000 slugs and 600 snails were collected from 157 localities in USA (Oregon, Colorado, Michigan, Wisconsin, Indiana, Ohio, Pennsylvania, Maryland, and New Jersey) and Canada (Ontario and British Colombia). Nine species of slugs, *Arion*

ater, *A. fasciatus*, *A. intermedius*, *A. hortensis*, *A. subfuscus*, *Deroceras lavae*, *D. reticulatum*, *Lehmannia valentiana*, and *Limax maximus* were found. All slugs were shipped from the collection sites along with plant material on ice-packs. Upon arrival in the laboratory, the slugs/snails were fed with carrots in Petri dishes lined with two layers of wet filter paper. The slugs were examined daily and the dead slugs were dissected to determine the presence of nematodes. Nematodes were recovered from 44 localities. They were: *Caenorhabditis elegans*, *C. formusana*, *C. remanei*, *Caenorhabditis* spp., *Curviditis* sp., *Cuticularia oxycera*, *Diplogaster ltheriteri*, *Diplogaster* spp., *Dolichorhabditis dolichura*, *Panagrolaimus* spp., *Rhabditella axei*, *Rhabditis* spp., *Rhabditophanes schneideri*, *Rhabditophanes* sp., *Saprorhabditis adentifera*, *Steinernema* sp., and *Xylorhabditis* sp. Only *C. remanei* was recovered from snails in all the three localities.

EVALUATION OF FOSTHIAZATE FOR MANAGEMENT OF *MELOIDOGYNE CHITWOODI* IN IDAHO POTATOES. **Hafez, Saad L., and P. Sundararaj.** Parma Research and Extension Center, 29603 U of I Ln, Parma, Idaho 83660.

A series of experiments were conducted to compare the efficacy of fosthiazate for the management of *Meloidogyne chitwoodi* with other nematicides in potatoes under field conditions. Application of fosthiazate at preplant or hilling (2.5 or 5 lb ai/A) or at both preplant and hilling (2.5+2.5 lb ai /A) significantly reduced the number of nematode infected tubers and increased potato yield compared to untreated control. Among treatments, the percent of infected tubers was lower at higher doses of fosthiazate alone (5lb ai/A) or with combination of Mocap and Temik. In a comparison the efficacy of two formulations of Fosthiazate (5G and 900 EC) at two application methods (broadcast and band incorporation) indicated that broadcast application of 12 lb ai/A yielded significantly more potato tubers and resulted in lowest number of infected tubers. Another study revealed that broadcast incorporation of fosthiazate (900 EC, 12 lbs/A) produced the lower infection of potato tubers than in furrow application.

RESISTANT REACTION OF ALFALFA CULTIVARS TO THE LESION NEMATODE *PRATYLENCHUS PENETRANS*. **Hafez, Saad L. and P. Sundararaj.** Parma Research and Extension Center, 29603 U of I Ln, Parma, Idaho 83660.

In two separate greenhouse experiments, 24 alfalfa cultivars were evaluated for their resistance to lesion nematode *Pratylenchus penetrans*, as compared to the susceptible cultivar Baker. Fresh and dry weights of shoots and roots, along with nematode populations in the root and soil were considered. In the first experiment (11 cultivars), total and root population in all the tested cultivars were significantly lower as compared to the cultivar Baker. In two cultivars, ZM 9421 and ZB 9546, fresh shoot weight was higher compared to the susceptible cultivar. In the second experiment, among the 13 cultivars tested, a reduction in nematode population, an increase in fresh and dry weight of root as well as fresh shoot weight was observed in ZC 9751A. In two other cultivars there was no change in nematode population, although fresh shoot weight and dry root weight increased.

OCCURENCE OF THREE POPULATIONS OF *PASTEURIA PENETRANS* ON *MELOIDOGINE* SPP. IN CHINA. **Han, P., and X. Gao.** Laboratory of Plant Nematology, South China Agricultural University, Guangzhou 510642, P R China.

The occurrence of *Pasteuria* has been rarely investigated in China. During 1998–99, more than one hundred root and soil samples were collected in various agricultural areas in the provinces of Guangdong, Hunan and Yunnan. Three populations of *P. penetrans* were found in Hunan and Guangdong. Two populations of *P. penetrans* were found on *Meloidogine javanica* on tobacco (*Nicotiana tabacum*) in Hunan, and one on *Meloidogyne arenaria* on citrus in Guangdong. No strains of *Pasteuria* were found in Yunnan. The *P. penetrans* populations from Hunan parasitised females and their endospores attached to *M. javanica* J2. The *P. penetrans* strain from Guangdong parasitized females and their endospores attached to males. The endospores of the *P. penetrans*

attached to 49% of *M. arenaria* J2 in the early spring in a naturally-infested citrus orchard in Guangdong.

OCCURRENCE OF A NEW SPECIES OF PLANT-PARASITIC CYST NEMATODE *(HETERODERA* SP.) ON *PANICUM COLORATUM*. **Handoo, Zafar A.[1] and I. K. A. Ibrahim.[2]** [1]USDA, ARS, Nematology Laboratory, Beltsville, MD 20705-2350 USA and [2]Department of Plant Pathology, Alexandria University, Alexandria, Egypt.

An undescribed plant-parasitic cyst nematode *(Heterodera* sp.) was found associated with Qasabagrass roots (*Panicum coloratum*) growing near a date palm in Alexandria, Egypt. It is characterized in having second-stage juveniles (J2) with body length of 516 um (485–550), stylet length of 23 um (22.5–23.5) with anchor-shaped knobs, lateral field with 3 lines, tail 62–71 um long, hyaline tail terminus 40 um (35–43); cysts are lemon-shaped, dark to light brown in color with an extensive sub-crystalline layer covering the entire cyst, cysts ambifenestrate, well developed underbridge with finger-like projections, bullae present, vulva slit measuring 44–48 um long. Males are absent and females have heavy punctations on the cuticle. Its relationship to H. graminophila Golden & Birchfield, 1972 and *H. leuceilyma* Di Edwardo & Perry, 1964 described from Florida and Louisiana, USA, are discussed. The present known distribution is restricted to Alexandria, Egypt. Its economic importance in rangeland grasses and cultivated crops such as rice is not known.

EFFECTS OF SEEDLING AGE AND TEMPERATURE ON THE RELATIONSHIP BETWEEN INOCULUM DENSITY OF THE NEEDLE NEMATODE *LONGIDORUS AFRICANUS* AND DAMAGE TO CARROT. **Huang, Xiang, and A. T. Ploeg.** Department of Nematology, University of California, Riverside, CA 92521.

The needle nematode *Longidorus africanus* causes damage to carrot in the Imperial Valley of southern California. Plants are generally affected in the seedling stage, resulting in carrot forking or reduced fresh weight of the tap root. To predict risks of crop damage, it is necessary to understand the relationship between *L. africanus* population levels and plant growth. We have examined this relationship under the influence of seedling age (0, 10, 20 30 days after seeding) at time of exposure to 0–200 nematodes per plant (200 ml soil), and under the influence of soil temperature (17, 20, 25, 30 C) at 0–200 nematodes per plant (60 ml soil). The fresh weight and length of carrot tap roots were determined 60 days post inoculation, and were expressed relative to the non-inoculated controls. Data were fitted to a Seinhorst curve and relative minimum yields and tolerance levels were estimated. The minimum relative carrot weight was not affected by temperature, whereas temperature did affect the minimum relative tap root length: under the highest inoculum densities the reduction in the length of the tap root was less at 17 C than at the other temperatures. Tolerance levels were higher when soil temperatures were low, but even at the lowest temperature carrot growth was dramatically reduced at relatively low nematode inoculum levels. Delaying the time at which the seedlings were exposed to the nematodes increased both the tolerance of the seedlings to the nematodes and their minimum yields.

EFFECTS OF TALL FESCUE AND PERENNIAL RYEGRASS CULTIVARS ON *PRATYLENCHUS NEGLECTUS* AND *PARATRICHODORUS ALLIUS*. **Ingham,[1] R. E., J. P. McMorran,[2] and N. M. Wade.[1]** [1]Dept. of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331-2902, and [2]Dept. of Crop Science, Hermiston Agriculture Research and Extension Center, Oregon State University, P.O. Box 105, Hermiston, OR 97838.

Ten cultivars each of tall fescue and perennial ryegrass were planted in field plots containing *Pratylenchus neglectus* and *Paratrichodorus allius*. Wheat and oats were included as known host crops. One year after planting (13 October, 1999), all fescue cultivars had fewer *P. neglectus* than wheat or oats. Wheat and oats averaged 249 and 105/250 g soil, respectively, and Wolf Pack and Tan Fescue averaged 8 and 7/250 g soil, respectively. All ryegrass cultivars were poorer hosts than wheat, but only cultivar 5813 had fewer *P. neglectus* (14/250 g soil) than oats. Only three fescue

cultivars, Au Triumph, Barlexus and Martin II, had fewer *P. allius* than wheat and only Martin II had a fewer than oats. Final densities in wheat, oats, Au Triumph, Barlexus, and Martin II were 17, 10, 5, 5 and 2/250 g soil, respectively. Most ryegrass cultivars supported fewer *P. allius* than wheat, but only three cultivars had fewer *P. allius* than oats. Navajo, Bright Star II and Stardance averaged 3, 3 and 2 *P. allius*/250 g soil, respectively.

A NEW SPECIES OF *PRATYLENCHUS* FROM CITRUS IN BRAZIL. **Inserra, R. N. ,[1] L. W. Duncan,[2] A. Troccoli,[3] J. Maia dos Santos,[4] and N. Vovlas.[3]** [1]Florida Department of Agriculture & Consumer Services, DPI, Gainesville, FL 32614-7100, [2]University of Florida, CREC, Lake Alfred, FL 33850, [3]Istituto Nematologia Agraria, CNR, 70126 Bari, Italy, and [4]UNESP/Dep. Entomologia e Nematologia, 14870.000 Jaboticabal SP, Brazil.

A recent molecular and morphological study of lesion nematodes similar to *Pratylenchus coffeae* revealed significant variation between a topotype population of *P. coffeae* from coffee in Java and a population of putative *P. coffeae* collected from citrus in Brazil. The population from Brazil shares similar head morphology with *P. coffeae,* having all labial sectors fused together and at least partial fusion with the oral disc. The mean values of stylet length ($\leq$ 15.5 μm vs. $\geq$15.5 μm), stylet knob height ($\leq$ 2.7 vs. $\geq$2.7), and vulva position ($\leq$79% vs. $\geq$79%) are smaller for the species from Brazil than for *P. coffeae.* The tail is subhemispherical with a smooth terminus in the *Pratylenchus* from Brazil, whereas it is commonly truncated and indented in *P. coffeae.* Discriminating between the two species requires several specimens because the diagnostic morphometric characters overlap slightly and because aberrant tail shapes exist in each species.

PHYSIOLOGICAL AND BEHAVIORAL DIFFERENCES BETWEEN KOREAN AND AMERICAN STRAINS OF *STEINERNEMA GLASERI*. **Itou, K.** Crop Nematode Laboratory, National Agriculture Research Center, Tsukuba, Ibaraki 305-8666, Japan.

Some physiological, morphological and behavioral characters were compared between three Korean isolates and eight US isolates of *Steinernema glaseri*. Isozyme and total protein patterns of infective juveniles were examined by polyacrylamide gel electrophoresis. Regarding esterase, *S. glaseri* had six isozymes and all the US isolates had at least one common band (Rm = 63 at 5-20% gradient gel). Korean isolates did not have this band, thus they were easily distinguished from US isolates. Total protein patterns were also different between Korean and US isolates, while US isolates showed similar patterns. Among Korean isolates, two isolates showed similar pattern of esterase and total protein. Motility of infective juveniles was evaluated on 1% agar. Korean isolates moved more actively than US isolates; average distance moved within 2 hour was 6.8 mm for Korean and 3.3 mm for US isolates. Infective juveniles of Korean isolates were smaller than US isolates; body length of the Korean and US isolates were 906 micro m and 1124 micro m respectively. Different color of *Galleria mellonella* cadaver infected by Korean and US isolates (beige by Korean, dark brown by US isolates) indicate that the strain of symbiotic bacteria is also different between Korean and US isolates. These observations suggest that Korean isolates of *S. glaseri* differ from US isolates in protein, morphology and behavior, and are possibly distinct strains.

PCR-RFLP-BASED SURVEY OF PLANT PARASITIC NEMATODES ASSOCIATED WITH SWEET POTATO AND TARO IN KYUSHU, JAPAN. **Iwahori, Hideaki,[1] Z. Sano,[1] and T. Ogawa.[2]** [1]Kyushu National Agric. Exp. Station, Kumamoto 861-1192, and [2]Nagasaki Pref. Plant Disease and Insect Control Station, Nagasaki 854-0062, Japan.

Meloidogyne spp., *Pratylenchus* spp., and *Rotylenchulus reniformis* in 85 sweet potato and 22 taro fields were surveyed and identified by PCR-RFLP analysis and nematode morphology. All nematodes were extracted with the Baermann funnel method. *Meloidogyne* spp. were detected in almost all sweet potato fields and 59% of the taro fields. The most prevalent species was *M. incognita.* There were few sweet potato fields with *M. arenaria* or *M. javanica.* The ratio of *M. incognita* to *M. arenaria* or *M. javanica* was about equal in taro fields. *Pratylenchus* spp., primarily

P. coffeae, were detected in 45% of the taro fields and 22% of the sweet potato fields. *Rotylenchulus reniformis* was isolated from 66% of the sweet potato fields and 32% of the taro fields. Amphimictic and parthenogenetic forms of *R. reniformis* were present and distinguishable by PCR-RFLP analysis.

INFECTION BEHAVIOR AND CONTROL OF FOLIAR NEMATODE *APHELENCHOIDES FRAGARIAE* INFESTING *HOSTA*. **Jagdale, G. B., and P. S. Grewal.** Department of Entomology, OARDC, Ohio State University, Wooster, OH 44691.

Foliar nematode, *Aphelenchoides fragariae* causes serious damage to *Hosta*, one of the most popular ornamental plants grown in urban landscapes. It has been reported that the infection of foliar nematodes in leaves occurs through stomata resulting in necrotic lesions limited by large veins. The present study compares the infection behavior of foliar nematodes cultured on *Rhizoctonia solani* and on live *Hosta* plants. Inoculation was performed on under- or upper-sides of non-injured or mechanically injured intact leaves on plants, using either nematode-infected *Hosta* leaves as a source of inoculum or nematodes recovered from the fungus. Both sources of nematode inoculum were pathogenic to *Hosta*. Injury on under- or upper-sides of the leaves appears to have no effect on nematode infectivity. Of 20 *Hosta* varieties tested, only four were resistant to foliar nematode infection. Thus, this study suggests that the major source of nematode infection in healthy *Hosta* leaves is through contact with infected leaves. Hot water and UV treatments were evaluated as potential curative measures. Exposure of infected leaves to UV (320 nm) for 4 h caused 100% nematode mortality. Hot water treatment for 10 min at 51 °C produced 100% mortality of nematodes in the leaves.

INDIRECT BENEFITS OF CONTROLLING *GLOBODERA TABACUM SOLANACEARUM* ON FLUE-CURED TOBACCO. **Johnson, C. S.** Southern Piedmont Agricultural Research and Extension Center, Virginia Tech, Blackstone, VA 23824.

Time to flowering was monitored in small-plot nematicide tests conducted in 1998 and 1999 to investigate the influence of controlling *Globodera tabacum solanacearum* on accelerating crop development and improving crop uniformity. In-row fumigation in 1998 increased percent flowering 9 weeks after transplanting from 19.8% for the untreated control to 49.3% for 84 liters/ha of 1,3-dichloropropene and a maximum of 96.7% for 75 liters/ha of chloropicrin. In-row fumigation in 1999 increased percent flowering 9 weeks after transplanting from 1.2% for the untreated control to 31.6% for 114 liters/ha metam-sodium and 90.9% for 74 liters/ha chloropicrin. In 1999, only 62.9% of plants in untreated soil had flowered 13 weeks after transplanting, while over 90% had flowered when planted into in-row fumigated soil. Broadcast fumigation reduced time to flowering compared to the untreated control , but was not as effective as in-row fumigation or use of Temik. In addition to improving yield and quality, control of *G. tabacum solanacearum* may reduce labor costs to tobacco producers and minimize delayed marketing of the crop. The economic benefits accruing from these labor savings and improved prices are significant and may partially explain hesitance by growers to adopt certain integrated pest management practices.

CHARACTERIZATION OF SECRETED PROTEINS OF *GLOBODERA ROSTOCHIENSIS*. **Jones, John T.,[1] V. C. Blok,[1] H. Popeijus,[2] L. Robertson,[3] A. Prior,[1] M. Phillips,[1] and G. Smant.[2]** [1]Unit of Mycology, Bacteriology and Nematology, Scottish Crop Research Institute, Invergowrie, Dundee, DD2 5DA, UK, [2]Laboratory of Nematology, Wageningen University, Binnehaven 10, 6709 PD Wageningen, The Netherlands, and [3]Universidad Autonoma de Madrid, Departmento de Biologia, Cantoblanco 28049, Madrid, Spain.

As techniques for large-scale DNA sequencing have become less expensive and more accessible, the challenge for molecular biologists has shifted from isolating potential genes of interest to performing functional analysis of cloned genes. We have isolated genes encoding a variety of secreted proteins including several antioxidant proteins (thioredoxin peroxidase, glutathione per-

oxidase and superoxide dismutase), a protein present in the secretions of the amphidial sense organs and homologs of surface-secreted proteins of animal parasitic nematodes. Biochemical studies have been used to characterize the functional properties of the secreted proteins. Our findings indicate that the thioredoxin peroxidase is the major hydrogen peroxide removal enzyme secreted by *G. rostochiensis*. Biochemical evidence suggests that the secreted glutathione peroxidase has a different functional role and is unlikely to be involved in protecting the parasite from host defense responses. We will also present in situ hybridization data showing spatial localization patterns of our genes.

CHARACTERIZATION OF A WATER SOLUBLE PHEROMONE COMPONENT FROM THE SUGAR BEET CYST NEMATODE, *HETERODERA SCHACHTII,* USING NOVEL AND CONVENTIONAL BIOASSAYS. **Jonz, M. G.,[1] E. Riga,[2] A. J. Mercier,[1] and J. W. Potter.[2]** [1]Dept. of Biological Sciences, Brock University, St. Catharines, Ontario, Canada, L2S 3A1, and [2]Agriculture and Agri-Food Canada, SCPFRC, Vineland Station, Ontario, Canada, L0R 2E0.

Females of the sugar beet cyst nematode, *Heterodera schachtii*, form sedentary cysts, while mobile males maintain a vermiform morphology and must seek out mates for amphimictic reproduction. Prior to copulation, male *H. schachtii* nematodes orient themselves toward the source of a putative pheromone emitted by homospecific females, and exhibit stomatostylet thrusting activity. Males displayed these typical mating behaviours in vitro in a concentration dependent manner upon exposure to crude and fractionated female conditioned medium (FCM) in Petri dish and stomatostylet activity bioassays. Males showed no response to male conditioned medium, unconditioned medium or vanillic acid, the suspected pheromone of *H. glycines*. The lack of response of juveniles to FCM indicates that the activity of FCM is specific to males. We demonstrate the activity of FCM in behavioural bioassays and report a novel method by which stimulatory substances, such as pheromones, may be assayed. We have characterized a water soluble pheromone component from *H. schachtii* and suggest that this substance is remarkably stable and is active at very low concentrations. Absorption spectrophotometry and reverse phase HPLC analysis allowed the isolation of a biologically active fraction of the crude FCM which contains a component of the female *H. schachtii* pheromone. This work may ultimately lead to the isolation of a pheromone and the use of this substance in a pest management strategy.

RETENTION AND RELEASE OF TOBRAVIRUS PARTICLES BY VIRUS VECTOR TRICHODORID NEMATODES. **Karanastasi, E., I. M. Roberts, S. A. MacFarlane, and D. J. F. Brown.** Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA, Scotland, UK.

Tobacco rattle tobravirus is transmitted by plant parasitic, soil inhabiting trichodorid nematodes. The nematodes acquire virus particles while feeding on roots of infected plants, and the virus becomes specifically retained along the nematode's pharyngeal tract from which it is subsequently released to infect further plants. Electron microscopy study on serial ultrathin sections of several trichodorid virus-transmitting species has revealed that differences may occur in the nature of retention and release of virus particles from the vectors. Virus particles present in the pharyngeal tract, anterior to the outlet of the dorsal gland, may not necessarily be retained and are washed into the plant cells, most likely along with secretions deriving from the dorsal gland. Virus particles present in the esophageal bulb are persistent and retained on a long-term basis, can be slowly released, and probably account for serial transmission of TRV to several plants.

ANALYSIS OF *GRP-GUS* FUSIONS IN *ARABIDOPSIS THALIANA* UPON NEMATODE INFECTION. **Karimi, M.,[1] C.-L. Manes,[1] M. Van Montagu,[1] and G. Gheysen.[1,2]** [1]Vakgroep Moleculaire Genetica, Departement Plantengenetica, Vlaams Interuniversitair Instituut voor Biotechnologie, Universiteit Gent, K.L Ledeganckstraat 35 B-9000 Gent, Belgium, and [2]Vakgroep Moleculaire Biotechnologie, Faculteit Landbouwkundige en Toegepaste Biologische Wetenschappen, Universiteit Gent, Coupure Links 653, B-9000 Gent, Belgium.

The putative *Arabidopsis* oleosin genes, *atgrp-6, atgrp-7,* and *atgrp-8* are expressed predominantly in the anthers, and more specifically in the tapetum layer. Tapetal cells are responsible for nutrition of developing pollen grains and show some functional similarities to nematode feeding sites (NFS) induced in plant roots by sedentary parasitic nematodes. The aim of this study was the expression analysis of tapetal specific genes in NFS. We have analyzed expression of these *grp* genes upon infection with the root-knot nematode *Meloidogyne incognita* and the cyst nematode *Heterodera schachtii*. Glucuronidase (GUS) analyses were conducted three days, one week, two weeks, and one month post infection. The histochemical analysis showed upregulation of GUS only three days after inoculation and a maximal GUS staining was seen one week after infection.

USE OF FLUORESCENT *PSEUDOMONAS* SPP. TO INDUCE RESISTANCE TO CLOVER CYST NEMATODE AND BLUE-GREEN APHID ON WHITE CLOVER. **Kempster, Valerie N., K. A. Davies, and E. S. Scott.** Department of Applied and Molecular Ecology, University of Adelaide, Glen Osmond, 5064, South Australia, Australia.

Isolates of soil bacteria were tested for their ability to induce resistance to pests of white clover. The experiments were conducted as a soil-based growth cabinet bioassay at 19 °C. Fluorescent *Pseudomonas* spp. were isolated from nematode-infested sites, and selected for ability to break down pectin. Pectinolytic strains P29 and P80 were applied as a root drench to white clover seedlings. Treatments with 2 ml of 50μM Benzo(1,2,3)thiadiazole-7-carbothioic acid-S-methyl ester (BTH) were set up as positive controls. Treatments were applied 1 to 3 days prior to inoculation with the clover cyst nematode *Heterodera trifolii*. After 4 to 5 weeks, treated plants had a higher proportion of distorted cysts and cysts with less than 50 eggs/cyst, compared to water-treated controls. Greenhouse tests of BTH and P29 against the blue-green aphid *Acyrthosiphon kondoi* on white clover also showed induced resistance.

MOLECULAR METHODS TO STUDY THE RHIZOSPHERE INTERACTIONS OF *VERTICILLIUM CHLAMYDOSPORIUM.* **Kerry, B. R., T. H. Mauchline, S. D. Atkins, K. G. Davies and P. R. Hirsch.** IACR-Rothamsted, Harpenden, Hertfordshire, AL5 2JQ, UK.

Observations on the saprophytic growth of *Verticillium chlamydosporium* through the use of a selective medium have been made, and relative changes in its abundance demonstrated the importance of rhizosphere colonization for control of root-knot nematodes. Changes in fungal biomass, physiological state and population genetics cannot be estimated from colony counts on selective media. More discriminatory methods are required to quantify and visualize the fungus in the rhizosphere. ERIC primers used in PCR reactions enable different isolates of the fungus to be reliably distinguished but this technique is suitable only for the amplification of DNA derived from cultures on agar. Primers designed to amplify a 270 bp segment of the *V. chlamydosporium* β-tubulin gene have been used to detect the fungus in DNA extracted from soil. Three monoclonal antibodies have been produced that enable the fungus to be visualized on the root. The fungus has been transformed to express the *gfp* gene but the lack of selectable markers to recover transformants has delayed progress in their use in ecological studies.

APPLICATION OF THE MATURITY INDEX AND ITS RELATIONSHIP TO SELECTED ENVIRONMENTAL FACTORS IN THREE TERRESTRIAL HABITATS IN BUTTERPOT PROVINCIAL PARK, NEWFOUNDLAND. **King, Ian Wm., and J. R. Finney-Crawley.** Department of Biology, Memorial University of Newfoundland, St. John's, NF, Canada, A1B 3X9.

Using data from a study of the nematode fauna of three terrestrial habitats, Maturity Index (MI) values were calculated. The three habitats (a black spruce-moss forest, a Dryopteris-white birch forest, and a dry Kalmia heath-barren) were all located in Butterpot Provincial Park (53°W, 47°30′N) on the Avalon Peninsula of Newfoundland. In the original study, collections were done at 6- week- intervals from mid-May until the beginning of November. Cores from the black spruce-moss and Kalmia heath-barren sites were divided into three subcores. The uppermost (FH)

contained both partly decomposed organic material (felt) and organic material whose original structures were unrecognizable (humus). The Ae horizon, a mineral layer near the surface, was characterized by the removal of either clay, iron, aluminum or organic material individually or in combination. The lowest layer (B) was a region of maximum accumulation of materials such as silicate clays and iron and aluminum oxides. In addition to these, the Dryopteris-white birch site included an uppermost litter (L) layer, in which original structures of the organic material were easily recognizable. For each soil horizon, at each site and for each sampling time, the MI value was calculated. Statistical analysis showed significant differences between MI values from different soil layers, different sampling times, and equivalent layers from different sites. The relationship between these differences and selected environmental factors are considered. These factors include subcore temperature, pH, moisture content, particle size, percentage of organic material, and available nitrogen, phosphorous, potassium, sodium, calcium and magnesium.

YIELD AND NEMATODE NUMBERS OF SELECTED SOYBEAN CULTIVARS IN A FIELD INFESTED WITH RENIFORM NEMATODES. **King, P. S.,[1] D. B. Weaver,[2] and R. Rodríguez-Kábana.[1]** Departments of [1]Entomology and Plant Pathology and [2]Agronomy and Soils, Auburn University, Alabama 36849-5409.

Twenty-eight soybean cultivars (*Glycine max*) were evaluated for resistance to *Rotylenchulus reniformis* in a field in central Alabama heavily infested with the nematode (>400/100cm3 soil The selected cultivars covered maturity groups ranging from V to VIII, and a wide range of known resistance and susceptibility levels to root-knot *(Meloidogyne* spp.) nematodes and SCN (*Heterodera glycines*). No cultivar tested showed high resistance to *R. reniformis*. Three cultivars (Motte, Stonewall, and Boggs) showed moderate resistance (70–100 nematodes/100cm^3 soil), while three others (DP7375RR, Maxcy, and Carver) showed moderately low resistance (100–200 nematodes/100cm^3 soil). All other cultivars tested showed little or no resistance (250–800 nematodes/100cm^3). Very little correlation could be determined between nematode populations and yield response.

CHARACTERIZATION AND USE OF A SOIL-BORNE BACTERIUM TO CONTROL THE PHYTOPARASITIC NEMATODE, *MESOCRICONEMA XENOPLAX*. **Kluepfel, Daniel,[1] A. Nyczepir,[3] J. Lawrence,[1] T. McInnis,[2] E. Zehr,[1] B. Glandorf,[1] P. Wechter,[1] B. Leverentz.[1]** [1]Department of Plant Pathology and Physiology, and [2]Department of Biological Sciences, Clemson University, Clemson, SC 29634, and [3]USDA, Southeastern Fruit and Tree Nut Research Laboratory, Byron, GA. 31008.

The ring nematode, *Mesocriconema xenoplax*, is an ectoparasitic migratory nematode that has been shown to induce Peach Tree Short Life (PTSL) in the southeastern US. Seven fluorescent *Pseudomonas* spp. capable of inhibiting M. *xenoplax* multiplication have been isolated from soil sites that suppress PTSL and ring nematode multiplication. One of these seven, *Pseudomonas* spp. BG33R inhibits M. *xenoplax* multiplication in vivo and egg hatch in vitro. *M. xenoplax* populations on peach seedlings inoculated with BG33R and planted into solarized field plots remained at or below detection limits for nearly 18 months post- inoculation and solarization. Soil solarization alone also generated a microbial community suppressive to the development of the ring nematode. *M. xenoplax* populations on uninoculated trees planted into unsolarized soil reached levels of approximately 500 nematodes/100cm^3 soil. Five Tn5 egg-kill negative mutants of BG33R have been generated. The Tn5 insertion site in each mutant has been cloned and sequenced. DNA sequence analysis has revealed a high degree of homology to several genes of interest that will be discussed in relation to their potential involvement in egg-kill factor (ekf) production. The BG33R Tn5 ekf negative mutants are also protease and salicylic acid negative and express twice the amount of fluorescent siderophore as the wild type parent. The possible commercial use of BG33R in the control of ring nematodes in peach orchards will be discussed.

COTTON RESISTANCE AND TOLERANCE TO *MELOIDOGYNE INCOGNITA* AND *ROTYLENCHULUS RENIFORMIS* IN NORTH CAROLINA. **Koenning, S. R.,[1] K. R. Barker,[1] and D. T. Bowman.[2]** [1]Department of Plant Pathology, North Carolina State University, Raleigh, NC 27695-7616, and [2]Department of Crop Science, North Carolina State University, Raleigh, NC 27695-8604.

The reproductive and damage potential of the southern root-knot nematode, *Meloidogyne incognita*, and the reniform nematode, *Rotylenchulus reniformis*, on cotton were evaluated in field experiments. A split plot design was used to evaluate cultivars or breeding lines as whole plots. Fumigation with or without 1,3-dichloropropene was used as subplots. Susceptible cotton cultivars Deltapine (DP) 50, DP 90 and Suregrow 125 were compared to resistant cotton cultivars Paymaster H 1560, Stoneville LA 887, and CPCSD Nem-X in three fields infested with *M. incognita*. Fumigation generally increased the yield of both resistant and susceptible cultivars in fields infested with root-knot nematode. Breeding lines potentially tolerant or resistant to *R. reniformis* were compared to two standard cotton cultivars, DP 50 and LA 887, in a field naturally infested with this nematode. Numbers of *R. reniformis* were suppressed at mid-season, and cotton-lint yield was increased by fumigation with 1,3-dichloropropene. The putatively tolerant breeding lines tended to support lower levels of *R. reniformis* reproduction than did the two standard cultivars. The breeding lines had higher tolerance indices to reniform nematode than the standard cultivars used in this research, but the yields were lower than the standard cultivars. Fumigation resulted in a 100 to 200 kg/ha increase in cotton lint yield for cultivars LA 887 and DP 50 in the presence of *R. reniformis*.

EFFICACY OF PLANTPRO 45 AS AN ALTERNATIVE TO METHYL BROMIDE FOR CONTROL OF ROOT-KNOT NEMATODE ON TOMATO. **Kokalis-Burelle, N.[1] and P. Fuentes-Borquez.[2]** [1]USDA, ARS, U.S. Horticultural Research Lab, Ft. Pierce, FL 34945, and [2]Ajay North America, LLC, Powder Springs, GA 30127.

PLANTPRO 45, an iodine based compound, was evaluated as an alternative to methyl bromide for control of the root-knot nematode on tomato in Florida. In vitro, PLANTPRO 45 at 60 ppm reduced root-knot nematode egg viability by 75% compared to the untreated control. In greenhouse experiments PLANTPRO 45 significantly reduced gall formation on tomato at rates of 60–120 ppm. Fall 1999 field trials in Sanford, Florida were designed to optimize methodology for application through drip irrigation. Results indicated that two drip lines/0.9 m-wide bed provided adequate dispersal of material and resulted in significantly less galling than one central drip line. Application through two drip lines at 120 ppm preplant and 80 ppm postplant, 17 and 35 days after planting, provided levels of control equivalent to fumigation with methyl bromide (448 kg/ha) in soils containing up to 5000 juveniles/250 cc soil of *Meloidogyne* spp.

AENORHABDITIS ELEGANS AS A MODEL TO IDENTIFY BACTERIAL VIRULENCE FACTORS. **Kurz, C. Léopold, and J. J. Ewbank.** Centre d'Immunologie de Marseille-Luminy, INSERM/CNRS/Université de la Méditerranée, Case 906, 13288 Marseille, France.

Tan and Ausubel, working with the enterobacterium *Pseudomonas aeruginosa*, have established *Caenorhabditis elegans* as a model for the study of pathogenesis and host defenses. We have extended this work and demonstrated that *Serratia marcescens* is also capable of infecting *C. elegans.* The bacteria are able to survive within the usually hostile environment of the nematode intestine, proliferate, and kill the host. Under standard assay conditions, the progression of the infection is highly reproducible. This allows one to screen *S. marcescens* mutants for those showing reduced virulence. *C. elegans* can be cultivated in either 96-well microtiter plates or 24-well culture plates, and thousands of bacterial clones can be individually tested. Screens of a *S. marcescens* mutant library produced by transposon insertion (in collaboration with the group of B. Finlay) suggest that a significant number of genes are involved in virulence. The molecular characterization of these mutants may reveal novel virulence factors that represent potential drug targets.

ROLES OF SECRETED PROTEINS IN ROOT-KNOT NEMATODE PARASITISM. **Lambert, K. N.** Department of Crop Sciences, University of Illinois, Urbana, IL 61801.

Root-knot nematodes *(Meloidogyne* spp.) are obligate sedentary endoparasites of almost all cultivated plants. In order for these nematodes to complete their life cycle they must be able to penetrate the root and initiate and maintain plant feeder cells. These early stages of parasitism may be regulated in the nematode by three glandular cells in the esophageal region of the nematode. Using a novel method, we have cloned a number of genes expressed in nematode esophageal glands. Two types of gland-specific genes will be discussed: 1) a nematode chorismate mutase that may act inside the plant cell to manipulate plant metabolism, 2) a putative pectate lyase that may aid the nematode in root penetration. In our presentation, we will present RNA in situ hybridization and immunolocation data to confirm expression in the esophageal glands, and preliminary data for the effects of expressing such nematode proteins in plants.

THE EFFECTS OF INSECT-PARASITIC NEMATODES ON *PRATYLENCHUS PENETRANS* POPULATIONS IN STRAWBERRIES. **LaMondia, J. A.,[1] and R. S. Cowles.[2]** [1]Department of Plant Pathology and Ecology, and [2]Department of Entomology, The Connecticut Agricultural Experiment Station, Windsor CT 06095.

The effects of inundatory releases of entomopathogenic *Steinernema carpocapsae* and *S. feltiae* infective juveniles on lesion nematode (*Pratylenchus penetrans*) populations in strawberry roots were determined in field microplots and small plots. One strawberry crown (cultivar Honeoye) was transplanted into each of 96 15-cm-d microplots containing soil naturally infested with lesion nematodes. Insect-parasitic nematodes were applied as a soil drench around strawberry roots at rates of 7.4 or 14.8 billion per ha in either May or August in each of two successive years. Plots were irrigated with 2.5 cm water after drench application to move nematodes into the root zone. Crowns were removed from plots each October and lesion nematodes extracted from 2 g roots using a wrist action shaker. There were no significant differences in numbers of *P. penetrans* extracted from roots of plants drenched with water alone or with *S. carpocapsae* or *S. feltiae* nematodes (208, 177, and 219 per 2 g root in 1998, and 343, 264, and 349 per 2 g root in 1999, respectively). Both *Steinernema* spp. were also drenched around roots of strawberry crowns in small plots in soil naturally infested with *P. penetrans.* Insect parasitic nematodes were applied as above at the same rates and timings over two seasons and lesion nematodes extracted from 2 g roots as above after sampling in November of the second year. The application of *Steinernema* nematodes again had no effects on the number of *P. penetrans* extracted from strawberry roots. Lesion nematode extraction was 36, 42 and 46 per 2 g root for the water control, *S. carpocapsae* or *S. feltiae*, respectively. Inundatory application of entomopathogenic *S. carpocapsae* and *S. feltiae* nematodes does not appear to affect populations of lesion nematodes in strawberry.

FIELD RESPONSE OF SELECTED MID-SOUTH COTTON VARIETIES TO THE RENIFORM NEMATODE **Lawrence, G. W.,[1] K. S. McLean,[2] and S. M. Baird.[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.

Thirteen mid-South cotton varieties were evaluated for resistance and yield response 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 Population density of 12,000 nematodes /500 cm^3 across all varieties was recovered at planting. Cotton varieties varied in response to the aldicarb applications. Seed cotton yields were improved 38.1 to 1,197.7 kg seed cotton/hectare with the addition of aldicarb. None of the cotton varieties included in this test showed resistance to the reniform nematode. A lack of yield response with aldicarb may indicate a degree of tolerance to the nematode.

HOST RESPONSE OF SELECTED MID-SOUTH SOYBEAN VARIETIES TO THE RENIFORM NEMATODE. **Lawrence, G. W.,[1] K. S. McLean,[2] and S. M. Baird.[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-seven mid-South soybean varieties in maturity groups (MG) IV through VI were evaluated for resistance to the reniform nematode (*Rotylenchulus reniformis*). Included in the study were eleven MG IV, thirteen MG V and three MG VI varieties. Soybean plants were inoculated with 2,000 *R. reniformis*/plant and allowed to grow for 60 days in the greenhouse. At harvest, plant growth was measured and nematodes were extracted and counted. For each variety, resistance was calculated by dividing the number of eggs and vermiform stages of *R. reniformis* at harvest by the number of nematodes used as inoculum. Resistance to *R. reniformis* was found in five MG V varieties (Asgrow 5602, Delta and Pine Land 5354, 5644RR, 5806R, Hornbeck 5770) and one MG VI variety (Asgrow 6101). None of the MG IV varieties included in these tests showed resistance to this isolate of *R. reniformis*.

EFFECT OF ENTOMOPATHOGENIC NEMATODES ON MOVEMENT AND EGG PRODUCTION OF *MELOIDOGYNE INCOGNITA*. **Lewis, E. E., and E. E. Perez.** Entomology Department, Virginia Tech and SU, Blacksburg, VA 24061.

Movement of *Meloidogyne incognita* was evaluated in the presence of either frozen *Galleria mellonella*, or infected for 2, 4, 6, and 8 days with *Steinernema carpocapsae, S. feltiae, S. glaseri* or *Heterorhabditis bacteriophora*. Infected or frozen *G. mellonella* were placed at the edge of a petri dish containing agar. One hour later *M. incognita* juveniles (J2) were released in the center of the dish. After three hours, J2 were counted on each half of the dish. Fewer J2 were found on the side of the dish containing infected *G. mellonella*. Frozen *G. mellonella* did not affect J2 movement. To test the effect of entomopathogenic nematodes on egg production of *M. incognita*, tomato seedlings (i.e. 10 cm tall) were inoculated with either 150 eggs of *M. incognita*, 150 eggs of *M. incognita* + 829 *S. feltiae* J3, or 150 eggs of *M. incognita* + 829 *H. bacteriophora* J3. Two weeks after inoculation, roots were stained and nematodes inside the roots were counted. Six weeks after inoculation, *M. incognita* eggs were extracted from the roots and counted. There were no significant differences among the treatments in the number of nematodes inside the roots. Roots inoculated with *S. feltiae* and *H. bacteriophora* had fewer *M. incognita* eggs than roots inoculated with *M. incognita* alone.

TRAFFICKING OF CARBON BASED PROBES ACROSS THE EGGSHELL OF *HETERODERA GLYCINES:* A MOLECULAR INTERSTATE. **Low J., and R. I. Bolla.** Department of Biology, Saint Louis University, St. Louis, MO 63103-2010.

Parasitism by soybean cyst nematode (SCN), *Heterodera glycines*, is responsible for the largest percentage of soybean crop yield loss. With the decreasing efficacy and availability of nematicides come neoteric ways to manage the pest. A better understanding of eggshell structure may lead to a better understanding of embryogenesis, hatching, resistance to dessication, and diapause of SCN, areas of the SCN which are potential targets for management strategies. If the permeability of the egg shell and the effects of hatching factors on the induction of ion or water channels can be understood, then it should be possible to transport materials into the developing egg. Thus, we proposed that one approach to management would be to disrupt the selective permeablity of nematode eggshell membranes. We identified various carbon based probes that were either taken in or excluded by the eggshell membranes. This molecular highway of sorts has several possible roles in the induction and prevention of SCN hatch.

CONCEPT, COMPONENTS AND STRATEGIES OF SOIL HEALTH. **Magdoff, F. R.** Department of Plant and Soil Science, Hills Building, University of Vermont, Burlington, VT 05405.

In the context of agricultural issues, soil health refers to the suitability of soil for the purposes

of plant production. It encompasses a wide variety of biological, chemical, and physical properties such as soil pH, nutrient levels, diversity of soil organisms, degree of compaction, water infiltration rates, and water storage ability, etc. Although a number of researchers have attempted to derive a soil quality (or health) index, the best use of the concept is probably in helping farmers improve management practices. Practices that improve soil health include the following: soil organic matter management to increase the active (particulate) fraction, using a variety of organic residues, better rotations, intensive use of appropriate cover crops, (re)integrating animal agriculture with crop farms, management for enhanced tilth and reduced compaction, reduced tillage, more attention to soil moisture status when traveling on the field, controlled traffic, and nutrient management that satisfies both plant production and environmental goals. Farmers that are able to creatively combine a number of practices that enhance soil health find that they have healthier crops and lower pest pressures.

INTERACTIONS BETWEEN WOOD-ROTTING BASIDIOMYCETES AND THE PINEWOOD NEMATODE ON AGAR AND IN WOOD. **Mamiya, Y.** Department of Agriculture, Tamagawa University, Tokyo, 194-8610 Japan.

Thorne and Barron (1984) reported that several species of wood-rotting fungi were capable of destroying nematodes. The ability of the fungi, *Pleurotus ostreatus, P. pulmonarius, P. eryngii, Lentinus edodes, Neolentinus lepideus, Cryptoporus volvatus, Trichaptum abietinum* and *Pholiota nameko* to destroy the pinewood nematode, *Bursaphelenchus xylophilus* was investigated. Approximately 200 pinewood nematodes were inoculated onto an agar petri plate as soon as a thin mat of sparse hyphal growth had radiated from the inoculation point through the adjacent agar. Pinewood nematode quickly became paralyzed when cultured on the first four species of fungi. Within 24 to 48 hours of exposure, almost all nematodes were penetrated and digested by fungal hyphae. The other four species of fungi were less effective in destroying the nematodes. After several days some of nematodes, however, were entrapped by the fungal hyphae. The results of multiplication tests of the pinewood nematode on hyphal mat growing on potato dextrose agar supported observtions of the nematode destroying capability of each fungus. Co-inoculation of *P. ostreatus* and the pinewood nematode into pine logs demonstrated that the presence of *P. ostreatus* significantly reduced numbers of *B. xylophilus* present in the inoculated logs. This result suggested that the fungus was capable of destroying nematodes in wood.

ECONOMIC CONSIDERATIONS FOR THE ADOPTION OF TRANSGENIC CROPS: THE CASE OF BT CORN. **Martin, Marshall A.** Department of Agricultural Economics, Purdue University, West Lafayette, IN 47907-1145.

The 2000 crop year is the fifth season for the planting of several transgenic crops in the United States. In 1999, transgenic crops represented 30%, 57%, and 50% of the U.S. corn, soybean, and cotton acreage, respectively. While the share of transgenic crop acres is expected to remain about the same in 2000 for soybeans and cotton, transgenic corn acreage is expected to decline. To adopt a transgenic crop, growers must consider several agronomic, pest management, production cost, grain handling, and marketing issues. A relatively high per acre technology fee, expected low European corn borer infestation levels in much of the Eastern Corn Belt, a refusal by several major food processors (e.g., Gerbers, Frito-Lay, A.E. Staley, National Starch) to purchase genetically modified corn, and an unwillingness by some European and Asian importers to purchase transgenic corn and by-products is largely responsible for the modest adoption of Bt corn in the United States. This paper provides detailed economic analysis of the adoption and refuge management considerations faced by U.S. corn producers. Unless expected European and southwestern corn borer infestation levels exceed about 40%, under current commodity price and normal yield conditions, it is not profitable for farmers to adopt Bt corn. If they do adopt Bt corn, under the U.S. Environmental Protection Agency guidelines they must plant at least a 20% refuge to non-Bt corn. This

strategy may involve modest additional planting costs and delays depending on the field size and the configuration of the refuge.

*RME*1 IS AN ESSENTIAL COMPONENT OF THE *MI-1*-MEDIATED ROOT-KNOT NEMATODE RESISTANCE PATHWAY IN TOMATO. **Martinez de Ilarduya, Oscar, and I. Kaloshian.** Department of Nematology, University of California, Riverside, CA 92521.

The tomato gene *Mi-1* confers resistance to three species of root-knot nematodes (RKNs; *Meloidogyne* spp.) and to the potato aphid (*Macrosiphum euphorbiae*). As an approach to elucidating the *Mi-1*-mediated resistance pathway, we screened for mutants showing susceptibility or reduced resistance to RKNs. After screening 972 M2 tomato families, we identified four mutants showing different degrees of susceptibility. One mutant, *rme1* (for resistance to *Meloidogyne*), isolated from a fast-neutron-irradiated seed, showed complete susceptibility. DNA blot hybridization data showed that this mutant has no detectable deletion in *Mi-1* or related genes. RT-PCR experiments indicated that *Mi-1* is expressed in rme1 roots and leaves. Crosses with both susceptible and resistant cultivars resulted in resistant F1 progeny, indicating that the mutation is in a distinct locus from *Mi-1*. Analysis of the F2 segregating population showed that the phenotype is due to a single recessive gene mutation. We currently are studying the rme1 response to the potato aphid, different *Meloidogyne* species, and to *Mi-1*-virulent RKNs. The wildtype parent of *rme1*, Motelle, contains additional resistance (R) genes conferring resistance against fungal pathogens. We are determining whether *rme1* is compromised in these R-gene mediated resistances. In addition to *rme1*, we have isolated three other mutants with reduced nematode resistance which are being analyzed.

EVIDENCE FOR INDEPENDENT GENETIC CONTROL OF REPRODUCTION AND GALLING TO *MELOIDOGYNE JAVANICA* IN LIMA BEAN. **Matthews, W. C. Jr.,[1] D. M. Helms,[2] and P. A. Roberts.[1]** [1]Department of Nematology, University of California, Riverside, CA 92521-0415, and [2]Deparment of Agronomy & Range Science, University of California, Davis, CA 95616-8515.

Multiple field screenings of over 130 accessions from a lima bean (*Phaseolus lunatus*) plant introduction (PI) collection identified 36 accessions that had low galling responses to both *Meloidogyne javanica* and *M. incognita*. In a confirmatory greenhouse test in pots, one accession (PI 256874) exhibited low galling and a high level of resistance to both root-knot species. This is the first report of resistance to reproduction of *M. javanica* in lima bean. Analyses of F_1, F_2, and F_2 −derived F_3 families of a cross between PI 256874 and a susceptible commercial lima bean (cv. 'Henderson') tested with *M. javanica* indicated the presence of two recessive genes. One of the genes was found to confer resistance to nematode reproduction. The second gene was found to confer resistance to the root-galling reaction, but had no effect on reproduction. Analysis of segregating populations also revealed that the two genes are inherited independently.

SPIRAL NEMATODE (*HELICOTYLENCHUS*) POPULATION DENSITY CORRELATED TO SOYBEAN FIELDS DISPLAYING ''PLATTE VALLEY YELLOWS.'' **May, N. R.** Department of Biology, Nebraska Wesleyan University, Lincoln, NE 68504-2796.

High densities of the plant-parasitic nematode genus *Helicotylenchus* are believed to be one causative agent of symptoms known as ''Platte Valley Yellows,'' which are seen in soybeans grown in the Nebraska River Valleys. Soil samples were taken on two occasions from eleven soybean fields exhibiting the yellowing symptoms and twelve soybean fields presenting no symptoms. Adjacent corn fields were also sampled for assessment purposes. A centrifugation technique was used to extract the nematodes. The mean for the first sampling of symptomatic soybean soil was 381.8(± 497) *Helicotylenchus*/100 cm^3 soil compared to 680(±679) for the second sampling. The first sample mean in the unaffected fields was 147(±271.6) compared to the second sample mean of 111(±183.4). The mean from the corn adjacent to the affected soybeans was 173(±207.5)

for the first sample and 319(±266) for the second sample. The mean from the corn adjacent to the unaffected soil was 155(±217.7) and 182(±360.0) for the first and second samples, respectively. The mean percent increase in the number of *Helicotylenchus* from the first sample to the second sample was 759%(±1525) in the fields showing the yellowing symptoms compared to a mean increase of 20%(±142.7) in the unaffected soybean fields. These findings support the claim that the population densities of *Helicotylenchus* increase throughout the growing season and may be linked to the ailing soybeans.

PROGRESS TOWARD HIGH THROUGHPUT GENE DISCOVERY IN PARASITIC NEMATODES; INITIAL FINDINGS FROM *MELOIDOGYNE INCOGNITA* EST SEQUENCING. **McCarter, James.,[1] D. Bird,[2] U. Rao,[2] A. Kloek,[3] S. Clifton,[1] D. Pape,[1] J. Martin,[1] T. Wylie,[1] S. Eddy,[1] R. Waterston,[1] and the Washington University GSC EST Team.[1]** [1]Genome Sequencing Center, Washington Univ. School of Medicine, St. Louis, MO 63108, [2]Department of Plant Pathology, North Carolina State Univ., Raleigh, NC 27695-7616, and [3]Divergence LLC, c/o Dept. of Genetics, Washington University School of Medicine, St. Louis, MO 63110.

To build upon knowledge gained from the complete genome sequence of *C. elegans*, the Genome Sequencing Center (St. Louis) and the Sanger Centre (Hinxton, UK) have recently begun high throughput Express Sequence Tag (EST) sequencing from parasitic nematodes. Funded by NIH-NIAID and the Wellcome Trust, this project will generate >200,000 ESTs over the next 3 years, sampling from 15 parasitic nematode species, including *Meloidogyne incognita* (Root Knot Nematode, RKN) and *Heterodera glycines* (Soybean Cyst Nematode). We anticipate the identification of 70–80,000 new nematode genes. We have begun pilot EST sequencing from a *M. incognita* J2 cDNA library. We will report on our progress analyzing the sequenced ESTs, including the creation of a RKN Gene Index by EST clustering and consensus sequence generation, identification of common and rare J2 transcripts, and identification of RKN–*C. elegans* orthologs. All sequences are available at www.ncbi.nlm.nih.gov/dbEST. Project progress will be reported at http://genome.wustl.edu/gsc.

POPULATION DYNAMICS OF *HETERODERA GLYCINES* ON SOYBEAN AT PARANAPANEMA VALLEY, SP, BRAZIL. **Mendes, Maria L.,[1] A. Jaehn,[2] L. C. B. Ferraz,[3] S. C. Costa,[1] J. E. Pereira,[4] and M. F. A. Silva.[2]** [1]UEL/Agronomia, Londrina, PR, Brazil 86051-990, [2]UNESP/FCA, Botucatu, SP 18603-970, [3]EALQL/Zoologia, Piracicaba, SP 13418-900, and [4]EMBRAPA, Londrina, PR 86001-970.

The effect of two cultural practices (fallow and corn cropping) and two soybean planting dates: normal (early November), and late (early January) dates on *Heterodera glycines* (SCN) was studied in a naturally infested field at Florinea, SP, Brazil. Soil samples were collected monthly during one year and the numbers of cysts, J2, and eggs were determined. The maximum multiplication rate and the probable number of nematode generations were also calculated. Data analysis did not detect correlation among different stages of the SCN life cycle and soil temperature or precipitation. Although increases were found in all treatments after soybean, the nematode population was significantly lower in the treatments in which soybeans were planted late after corn, indicating a lower multiplication rate. The probable number of generations was seven when soybean was planted in November and four when soybean planting was delayed until January.

WILL ANY TWO BIOLOGICAL AGENTS MANAGE ANY ONE NEMATODE? **Meyer, S. L. F.** USDA ARS Nematology Laboratory, Beltsville Agricultural Research Center, Beltsville, MD 20705-2350.

Many microbes are antagonistic to plant-parasitic nematodes, but few biocontrol agents have been marketed for nematode management. A major obstacle to the development of successful biocontrol formulations is inconsistent performance of applied organisms. One approach to this problem has been to study the efficacy of applying two potentially synergistic beneficial micro-

organisms. Such combinations may have greater ability to colonize the rhizosphere, express useful characteristics in a broader range of soil conditions, and have the added benefit of exhibiting antagonism to more than one plant pest or pathogen. Conversely, combined microbes may interact antagonistically with each other. Both results have occurred when two microbes have been applied jointly as biocontrol agents. Although this lack of predictability of joint application of biocontrol agents results in slow progress, the approach offers potential for overcoming some of the problems encountered with efficacy of individually applied microbes.

SOIL ECOLOGICAL CHANGES UNDER A LONG TERM CONSERVATION TILLAGE WHEAT AND MAIZE ROTATION TRIAL IN MEXICO. **Mezzalama, M., J. M. Nicol, K. Sayre, and P. Grace.** International Maize and Wheat Improvement Centre (CIMMYT).

Work has been conducted in 1997, 1998 and 1999 on an 8-year-long tillage (zero/conventional), rotation (wheat/maize) and residue (retained/removed) management trial in the central highlands initiated in 1991 at the CIMMYT experiment station of El Batan (2240 masl, average rainfall 600 mm) in Mexico. Wheat and maize yields, soil carbon and five soil microbial groups have been measured over years. The data from 1999 indicate that the root lesion nematode (*Pratylenchus thornei*) is significantly higher under continuous wheat rotation than with maize. Other non-parasitic nematodes and fluorescent Pseudomonads are significantly higher under straw retention than removal, as well as a 10% increase in soil organic carbon in the top 20 cm. Other soil microbial groups (*Fusarium* spp., total bacteria, total fungi, total bacteria and actinonomycetes) to date do not significantly differ with the various treatments implemented. The best crop management system involves growing maize and wheat in rotation using zero till and crops residue retention.

PATHOGENICTY AND VIRULENCE OF *MELOIDOGYNE HAPLA* POPULATIONS FROM VEGETABLES IN NEW YORK STATE. **Mitkowski, N. A., and G. S. Abawi.** Department of Plant Pathology, New York State Agricultural Experiment Station, Cornell University, Geneva, NY 14456.

Fourteen populations of *Meloidogyne hapla* were collected from the vegetable production regions within New York State. The pathogenicity of these populations was determined employing a modification of the North Carolina Differential Host Test. Virulence assays were performed on a subset of these populations using two varieties of susceptible lettuce (*Lactuca sativa*), 'Ithaca' and 'Montello'. Variation was observed in both pathogenicity and virulence between the different populations tested. In addition, interactions between nematode population and host variety were evident. Results of the pathogenicity and virulence tests, along with geographic origin, were correlated to results previously obtained from RAPD profiling and ITS sequences in order to assess whether these traits might be useful as genetic markers. ANOVA analysis was also used to identify the possible existence of sources of genetic variation among the populations.

EFFECTS OF FIELD APPLICATION METHODS OF *PASTEURIA PENETRANS* ON CONTROL OF *MELOIDOGYNE INCOGNITA* ON TOMATO. **Mizukubo, T., M. Talavera, K. Ito, and S. Aiba.** Crop Nematode Laboratory, Department of Plant Protection, National Agriculture Research Center, Tsukuba 305-8666, Ibaraki, Japan.

Application methods of *Pasteuria penetrans* (Pp) spores to field soil for biological control of *Meloidogyne incognita* on tomato were evaluated using two 3 × 3 factorial experiments in 2 × 2 m field plots. In the first experiment, the factors were application levels of Pp endospores (0, 2.5 × 109/m^2 and 5 × 1010/m^2) and a combination of post-application rotary plow (R) and watering at 10 L/m^2 drench (D) (0, R and R+D). In the second experiment, the factors were 40-cm-deep drill holes at (i) 40 cm apart, (ii) 20 cm distance between them and (iii) no drill holes and root enclosure cultivation with paper barriers set to cover large holes of 30-cm-deep, 30-cm-diam. which were refilled with steam sterilized soils (two levels: with or without barrier). In both experiments, the

factors did not affect the numbers of J2 per 20 g of soil nor Pf/Pi at harvest, 20 weeks after planting. Root gall indices were not affected by the factors in the first experiment, but were reduced ($P<0.05$) in the root enclosure trial of the second experiment. Rotary plow plus water drench 2 days after Pp incorporation into the soil increased Pp spore densities at harvest throughout every 10 cm layer (up to 40 cm) at both Pp application levels. Pp densities were also greater ($P<0.05$) at all the depths in the drill-hole plots than in non-drill-hole. Root enclosure did not reduce Pp densities inside or outside the paper barrier. The high Pp application level increased ($P<0.05$) the number of flowers in the third to seventh flower trusses, while post-application plow and drench did not affect them. Tomato yields in weight were greater at the higher Pp application level ($P<0.05$). The high Pp application level, drill-holes without plow or drench after Pp application contributed ($P<0.05$) to a greater number of large size fruits. There were no interactions between the factors investigated.

DISTRIBUTION OF *PARATRICHODORUS ALLIUS* AND TOBACCO RATTLE VIRUS IN PACIFIC NORTHWEST POTATO FIELDS. **Mojtahedi, Hassan,[1] G. S. Santo,[1] Z. Handoo,[2] J. M. Crosslin,[3] C. R. Brown,[3] and P. E. Thomas.[3]** [1]Washington State University, [2]USDA-ARS Beltsville, MD 20705-2350, and [3]USDA-ARS, 24106 Bunn Rd., Prosser, WA 99350-8694.

Soil samples from potato fields in Washington, Oregon and Idaho were collected, and the only stubby root nematode species identified was *Paratrichodorus allius.* The nematode was extracted and inoculated to Samsun NN tobacco to determine the presence of tobacco rattle virus (TRV). The pathogenic potential of TRV isolates was also determined on Russet Burbank and Norkotah potato using *P. allius* as vector. *P. allius* was found in 30% of potato fields, of which 10% contained viruliferous nematodes. Regardless of *P. allius* density, potato plant age or duration of exposure to the nematode, TRV from Pasco, WA, was the most virulent strain followed by Umatilla, OR, and Mattawa, WA.

PHYLOGENY OF *DORYLAIMIDA* INFERRED FROM PARTIAL 18S RDNA SEQUENCE DATA. **Mullin, P. G., A. L. Szalanski, T. S. Harris, and T. O. Powers.** Department of Plant Pathology, University of Nebraska-Lincoln, Lincoln, Nebraska 68583.

The *Dorylaimida* are the dominant order of terrestrial nematodes in both taxonomic diversity and sheer numbers, yet few phylogenetic studies have been undertaken to elucidate evolutionary relationships in this group. We present a preliminary phylogeny of dorylaimid nematodes, together with representatives of related groups, based on sequence data obtained from approximately 600 bp of the 18S ribosomal DNA repeat. This study reveals some points of departure from current classifications of *Dorylaimida,* including the apparent paraphyly of the superfamilies *Tylencholaimoidea* and *Belondiroidea. Longidoroidea, Dorylaimoidea,* and *Nygolaimoidea,* on the other hand, form well-supported clades. Within the *Dorylaimoidea,* genera such as *Labronema* and *Ecumenicus,* currently placed in the Qudsianematidae, appear to be more closely related to the *Aporcelaimidae,* while *Nordiidae* and *Dorylaimidae* appear to be well-defined groupings. Additional taxa are being examined to provide clarification of uncertainly-resolved areas of this phylogeny, particularly with respect to the placement of the monotypic superfamiliy *Campydoroidea.*

SURVEY OF TALLGRASS PRAIRIE NEMATODES: RICH DIVERSITY IN A TEMPERATE ECOSYSTEM. **Mullin, P. G., A. L. Szalanski, B. Higgins, C. Kolm, and T. O. Powers.** Department of Plant Pathology, University of Nebraska-Lincoln, Lincoln, Nebraska 68583.

An ongoing survey of the nematode fauna associated with C3 and C4 grasses at the Konza Prairie LTER in Manhattan, Kansas has revealed the presence of at least 111 genera and 305 morphologically determined species of soil-inhabiting nematodes in a relatively small area of undisturbed native prairie. This total significantly exceeds previous reports from this site and from similar areas. Total nematode populations range from 321 to 2350 per 100 cm^3 of soil, with an

average of 802 per 100 cm^3. An average of 53 morphologically distinct forms is observed in a given 100 cm^3 soil sample, with a range of 32 to 64 forms. Of the 305 species observed to date, 100 (33%) have been detected in only one sample, while 247 (81%) have been found in 25% or fewer of the samples examined. Only one species, *Helicotylenchus platyurus*, has been found in all of the samples. At least 7 of the observed morphological species appear to be undescribed. Members of the Dorylaimida (36 genera, 121 species) and Tylenchida (29 genera, 100 species) are the most frequently encountered, although representatives of Aphelenchida, Araeolaimida, Chromadorida, Enoplida, Monhysterida, Mononchida, Rhabditida, Strongylida, Triplonchida, and Tripylida have also been observed. Predaceous and/or omnivorous nematodes (predominantly Dorylaimida) exhibit the greatest diversity, based on the number of genera recorded. Microbivorous nematodes (including Cephalobidae, Plectidae, and Rhabditidae) are relatively rare. Most morphological species are present in very low numbers (per 100 cm^3 soil), although high relative populations of some species (particularly Tylenchid herbivores) are sometimes observed.

MORPHOLOGICAL DIVERSITY OF THE CEPHALIC REGION IN SOME CEPHALOBIDAE FROM CALIFORNIA. **Mundo-Ocampo, M.,[1] J. G. Baldwin,[2] D. J. Bumbarger,[1] S. Nadler,[2] S. P. Stock,[2] I. De Ley,[3] and P. De Ley.[3]** [1]Department of Nematology, University of California, Riverside, CA 92521, [2]Department of Nematology, University of California, Davis, CA 95616-8668, and [3]Instituut voor Dierkunde, Universiteit Gent, Belgium.

Cephalobidae genera are typically diagnosed by characters present in the cephalic probolae, which are highly derived extracellular structures anterior to the buccal capsule. It is our intent to obtain an understanding of homology of probolae in these taxa at the ultrastructural level within the context of comparative developmental biology. Through a rigorous assessment of homology based on structural and developmental similarity, compared within a robust molecular phylogenetic framework, we hope to determine characters that can accurately represent monophyletic groups, identify new characters for phylogenetic analysis and gain an understanding of patterns in character evolution. SEM of the lip region of *Acrobeles, Acromoldavicus, Chiloplacus, Eucephalobus Metacrobeles, Nothacrobeles, Paracrobeles, Stegelletina* and *Zeldia* is illustrated and homologies of particular features are discussed.

POTENTIAL OF EXTRACTS OF NEEM, *AZADIRACHTA INDICA A. JUSS*, FOR THE MANAGEMENT OF THE BANANA PARASITIC NEMATODES. **Musabyimana, T.,[1] R. C. Saxena,[2] E. W. Kairu,[3] C. K. P. O. Ogol,[3] A. Bélanger,[1] G. Bélair,[1] and Z. R. Khan.[2]** [1]Agriculture and Agri-Food Canada, Saint-Jean-sur-Richelieu, Quebec, Canada J3B 3E6, [2]International Centre of Insect Physiology and Ecology, P.O. Box 30772 Nairobi, Kenya, and [3]Kenyatta University, Department of Zoology, P.O.Box 43844 Nairobi, Kenya.

Powdered neem seed and cake, containing 4,000 and 5,800 ppm azadirachtin, respectively, were compared with Furadan 5G (carbofuran) against *Pratylenchus goodeyi* Sher & Allen and *Meloidogyne* spp. attacking banana. Nematodes were extracted from roots at plant flowering time by a maceration sieving technique and ber counted. Root necrosis due to nematode damage was evaluated on a 0 to 4 scale. Results indicated that soil applications of neem material at 100 g/plant at planting and subsequently, at 4-month intervals, reduced the nematode population density by 70 to 90% and their damage by 90 to 100% which was comparable with Furadan, applied at 60g/ plant at planting and then at 6-month intervals. Fruit yield of the second crop was 1.5 times greater in neem-treated plants than with Furadan and the untreated banana plants, indicating the superiority of the application of neem extracts over the synthetic nematicide.

PREVALENCE RATE OF INTESTINAL PARASITIC NEMATODES OF DOMESTIC FOWLS IN URMIA, IRAN. **Naem, Soraya.** P. O. Box 1177, Department of Pathobiology, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran.

This study was carried out from October 1998 to September 1999 and 105 domestic fowls from 43 villages in Urmia city were examined. All worms of each bird were removed from the small intestine and the caecum. The nematodes were washed with saline, fixed with F.A.F. and cleared with lactophenol. The nematodes were mounted on slides for diagnosis with light microscope (LM). All birds were infected with one to three nematodes. The results indicated that 32.4% of the birds examined were involved with *Ascaridia galli*, 61.9% with *Heterakis gallinarum* and 32.4% with *Subulura brampti*.

SELECTION FOR VIRULENCE AGAINST THE RESISTANCE GENE MI-1 IN TOMATO FROM AVIRULENT LINES OF *MELOIDOGYNE* SPP. **Narabu, T.** Upland Agriculture Research Center, Hokkaido Natl. Agr. Exp. Sta. Memuro, Hokkaido 082-0071, Japan.

Selection for virulence against the resistance gene Mi-1 was demonstrated from fifteen single egg mass isolates of *Meloidogyne* spp., three lines of *M. incognita* (MI), three lines of *M. javanica* (MJ), and nine lines of *M. arenaria* (MA). Each line, taken from the various fields where resistant tomatoes had not been grown before, produced no egg masses on the Mi-1-dependent resistant cultivars (R-cv) at 25 °C (23–27 °C), but produced several egg masses at 30 °C (28–32 °C) in greenhouse. Ten virulent lines reproduced slightly on R-cv at 25 °C and were selected from the avirulent lines by propagating them for two to six generations on R-cv at 30 °C. When these selected lines were propagated on R-cv at 25 °C, the pathogenicity at each generation varied among the lines. The numbers of egg masses produced on R-cv by inoculation of 500 second-stage juveniles increased progressively in two lines of MJ. The numbers became similar or higher to those on the susceptible cultivars after 4th or 12th generation, respectively. On the other hand, one line of MI and five lines of MA retain low virulence (the numbers of egg masses on R-cv was lower than those on the susceptible cultivars) for 6 to 35 generations, a behavior that is still continuing. Two lines of MA have lost virulence on R-cv after the 5th or 8th generation. These results suggest that continuous planting of resistant cultivars under high soil temperature conditions may promote the occurrence of virulent lines of *Meloidogyne* against Mi-1, though the frequency of occurrence varies depending on nematode lines.

ROLE OF NEMATODES IN SOIL HEALTH AND THEIR USE AS INDICATORS. **Neher, D. A.** Department of Biology, University of Toledo, Toledo, OH 43606-3390.

Nematode communities (plant-parasitic and free-living), characterized by life history strategy (maturity) indices, were evaluated as measures of soil health. Based on variance component, power curve, and reliability analyses, maturity indices perform better than indices of trophic diversity, abundance or proportions of individual taxa. Maturity indices correlate negatively with nitrogen availability and positively with rates of decomposition, thus reflecting ecosystem function. Forage and pasture crops may serve as reference sites for monitoring soils associated with annual crops. The cumulative frequency distribution of index values for soils with perennial crops (*Medicago sativa* L., *Festuca arundinacea* Schreb.) reflects a more complex food-web and, thus, a less disturbed biological community than soils with annual crops (*Glycine max* (L.) Merr., *Zea mays* L., *Triticum aestivum* L.). In contrast, maturity index values are similar to soils managed by conventional or organic techniques, suggesting that cultivation disturbs soil communities more than chemical applications. Management practices often represent a confounded mixture of physical and chemical attributes that can be separated using canonical correspondence analysis. Net impacts of physical and chemical management on individual genera are either cumulative or canceled by opposite responses. Regional-scale studies across North Carolina and Nebraska suggest that assessments of soil health based on nematode communities can be implemented on a geographic resolution of 125,000–200,000 km^2 of land area. Studies of this scale require a minimum sample size of 25 fields with one composite soil sample analyzed per field to detect 10% change (with 0.8 power) between two time periods.

COULD NATURAL ABUNDANCES OF $d^{13}C$ AND $d^{15}N$ IN DIFFERENT POPULATIONS OF *XIPHINEMA DIVERSICAUDATUM* (NEMATODA: LONGIDORIDAE), AFTER FEEDING ON DIFFERENT HOST PLANTS, INDICATE ENVIRONMENTAL PRESSURES OR ADAPTIVE FEEDING STRATEGIES? **Neilson, Roy, and D. Brown.** Scottish Crop Research Institute, Dundee, DD2 5DA, Scotland, UK.

$^{13}C/^{12}C$ ($d^{13}C$) and $^{15}N/^{14}N$ ($d^{15}N$) natural abundances from eight geographically disparate populations of the longidorid nematode, *Xiphinema diversicaudatum*, within Europe were analysed to determine whether whole body $d^{13}C$ and $d^{15}N$ would alter when transferred from one plant host to another. *A priori* assumptions were that the populations of *X. diversicaudatum* would be isotopically similar based on $d^{13}C$ (similar ^{13}C sources from new plant host) and $d^{15}N$ (identical feeding strategy) values. With the exception of the population from Germany, all *X. diversicaudatum* populations, after feeding for 28 days on a new plant host, *Petunia hybrida*, were approximately 2 % more ^{13}C-depleted compared with nematodes from the original plant hosts. However, in contrast to previously published data for different *Xiphinema* spp., levels of whole body ^{15}N-enrichment of nematodes after feeding on *Petunia* were variable. The only known biological difference between each of the *X. diversicaudatum* populations, apart from their geographic location, was the cropping of the source sites. The German population that was less ^{13}C-depleted than all the other populations originated from an ancient Roman viticulture site where grapevines were grown on shale to the virtual exclusion of other plants. The four populations that exhibited >1.5 % ^{15}N-enrichment originated from arable and soft fruit (including a range of weed species) sites, whereas those populations that had little or no ^{15}N-enrichment originated from grassland sites comprising only a few grass species. Our data would suggest that the original environment had an underlying effect on the isotopic values of the different populations of *X. diversicaudatum.* However, the mechanism by which this occurs is unclear. One possible explanation is that the exposure to differing numbers of alternative host plants may have led these populations to evolve different adaptive feeding strategies and this was manifested when introduced to a new plant host.

MOLECULAR CHARACTERIZATION OF TEN SPECIES OF *STEINERNEMA* USING RDNA ITS SEQUENCES. **Nguyen, K. B., J. Maruniak, and B. L. Adams.** University of Florida, Entomology and Nematology Department, Gainesville, FL 32601-0620.

ITS regions of 10 *Steinernema* spp. were PCR-amplified, sequenced, aligned, and compared. Each species has a unique DNA sequence, varying from 916 to 1061 base pairs in length. When mapped, several restriction enzymes reveal species-specific restriction sites and fragment length polymorphisms. Phylogenetic analysis support a monophyletic genus and reveals well established pattern of historical lineage independence among species. Genetic, morphological, and morphometric variability is concordant with the best estimate of phylogenetic relationship within the group. The origins and maintenance of key character transformations such as body size of infective juveniles, spicule and gubernaculum size and shape, and some other important morphological characters are identified and discussed within a phylogenetic framework.

PLANT YIELD LOSS AND SUSCEPTIBILITY TO THE ROOT-LESION NEMATODE (*PRATYLENCHUS THORNEI*) ON WHEAT IN SONORA, MEXICO. **Nicol, J. M., and I. Ortiz-Monasterio.** International Maize and Wheat Improvement Centre (CIMMYT).

The root lesion nematode (*Pratylenchus thornei*) is an economic pathogen on wheat with a global distribution. Experiments were conducted in Mexico in 1999 and 2000 to establish the yield loss and plant susceptibility on a set of historical varieties released by CIMMYT. The varieties were planted in a field infested with *P. thornei*, and the fumigant Basamid was used as a control. Water availability was also investigated by simulating drought and full irrigation treatments. Results from 1999 demonstrate that with irrigation there was no significant yield loss. Under drought, grain yield loss varied significantly from 2 to 40% with no trend associated with year of variety release. Hence there has been no involuntary selection for tolerance. Baviacora 92 had the

highest tolerance (2% grain yield loss), while Seri 82 had the lowest tolerance (40% grain yield loss). Although tolerance exists in the germplasm, there is no source of resistance, as all lines were found to be as susceptible as the Australian variety Warigal.

PROGRESS IN BREEDING FOR RESISTANCE IN WHEAT AGAINST ROOT LESION NEMATODE AND CEREAL CYST NEMATODES FOR GLOBAL ENVIRONMENTS. **Nicol, J. M., [1] and R. Rivoal.[2]** [1]International Maize and Wheat Improvement Centre (CIMMYT), and [2]Institut National Recherche Agronomique (INRA).

It is recognized that the nematodes, CCN (Cereal Cyst Nematode, *Heterodera spp*.) and the RLN (Root Lesion Nematode, *Pratylenchus thornei*) are globally distributed and economically important nematodes on wheat. CCN is a complex pathogen with many species and pathotypes. Attempts have been made to breed resistance against both nematodes. The Iraqi wheat landrace, AUS4930 has a high level of resistance against RLN. Furthermore it has one of the highest resistance levels across a range of CCN populations collected from different locations compared to the known sources of resistance. However, the landrace has been found to segregate for phenotype. Sixteen re-selections of AUS4930 were screened against 3 CCN populations and 2 RLN, and indicate nematode resistance appears correlated with plant phenotype. Current breeding is using these elite re-selections and advancing them into high yielding global wheats. Double haploid populations have been developed from AUS4930 crosses to understand the genetic control of the resistance against RLN and CCN.

USE OF RISK ASSESSMENT IN DESIGNING A REGIONAL SURVEY OF *DITYLENCHUS DIPSACI* ON ALFALFA. **Niles, R. K.,[1] R. M. Reich,[2] B. F. Dudek,[1] J. E. Cipra,[1] and D. H. Wall.[1]** [1]Natural Resource Ecology Laboratory, and [2]Department of Forest Sciences, Colorado State University, Fort Collins, CO 80523.

We are using spatial statistics and a Geographic Information System (GIS) for the South Platte River basin in Colorado to assess the risk of supporting high populations of *Ditylenchus dipsaci* (alfalfa stem nematode) in irrigated alfalfa fields. Our 1998 survey of alfalfa fields in Weld County, Colorado revealed the large-scale distribution of D. dipsaci on the landscape. We regressed the geo-referenced survey data against more than 50 GIS data layers involving soil and land management variables. The resulting model of *D. dipsaci* risk included driving variables related to soil texture and moisture. The model showed that some areas of high risk in the county were not yet heavily infested with the nematode. The risk model developed for Weld County was projected onto irrigated cropland in the adjoining Larimer and Morgan Counties. Areas of potentially low, moderate and high risk were identified. In 1999 we used these risk ratings in surveying *D. dipsaci* on alfalfa. Forty percent of the samples were collected from Larimer County and 60% from Morgan County. We successfully apportioned approximately an equal number of samples among the risk classes in each county. The goal of this project is to develop a risk model for *D. dipsaci* on alfalfa throughout the South Platte River basin.

FUSARIUM EQUISETI SECRETES COMPOUNDS ANTAGONISTIC TO ROOT-KNOT NEMATODE, *MELOIDOGYNE INCOGNITA*. **Nitao, James K.,[1] S. L. F. Meyer,[1] W. F. Schmidt,[2] J. C. Fettinger,[3] and D. J. Chitwood.[1]** [1]USDA, ARS, Nematology Laboratory, and [2]Environ. Chem. Laboratory, Beltsville, MD 20705; [3]Dept. of Chemistry & Biochemistry, Univ. of Maryland, MD 20742.

Fungi isolated from soybean cyst nematode were examined for production of nematode-antagonistic compounds. *Fusarium equiseti* produced inhibitors of root-knot nematode (*Meloidogyne incognita*) egg hatch and juvenile mobility. Bioassay-guided fractionation of a culture broth extract identified two active compounds, the trichothecenes 4,15-diacetylnivalenol and diacetoxyscirpenol. Trichothecenes possess broad spectrum toxicity, limiting their usefulness as templates for control agents. A second fungus species is also being examined. An Amberlite XAD extract of

the culture broth was fractionated using medium-pressure chromatography (Toyopearl resin and silica gel) and reversed-phase HPLC. The identities of the nematode-antagonistic compounds from the second fungus species are being determined. Efforts to identify natural products from fungi associated with plant-parasitic nematodes can provide lead compounds for new control agents and increase our understanding of nematode-fungus interactions.

POPULATION DYNAMICS OF *HETERODERA GLYCINES* AND SOYBEAN YIELD IN NO-TILL AND TILLED PRODUCTION SYSTEMS. **Noel, G. R., and L. M. Wax.** USDA, ARS and Department of Crop Sciences, University of Illinois, Urbana, IL 61801.

The effects of no-till, conventional tillage, and crop rotation on soybean yield and population dynamics of *Heterodera glycines* were compared during a 5-year study. Either *H. glycines*-resistant Linford soybean or susceptible Williams 82 soybean was rotated with corn and grown on 76-cm-wide rows in both tillage systems. Soybean was planted in 1994, 1996, and 1998. Soybean yield was not affected by tillage in any of the 3 years. Yield of Linford was greater than Williams 82 in all 3 years. In years in which soybean was planted, numbers of eggs/250 cm^3 soil increased more on both cultivars in no-till than in the conventional system. The Pf/Pi ratios on Williams 82 in the no-till system were 29, 125, and 86 and for Linford the ratios were 5, 40, and 79 for 1994, 1996, and 1998, respectively. However, crop rotation with corn negated these population increases so that numbers of eggs at planting were similar for each cultivar each year that soybean was planted.

EFFECTS OF CONTINUOUS CULTURE OF A RESISTANT TOMATO CULTIVAR ON *MELOIDOGYNE INCOGNITA* SOIL POPULATION DENSITY, AND PATHOGENICITY. **Noling, J. W.** University of Florida, IFAS, Citrus Research and Education Center, Lake Alfred, FL 33850.

Six field microplot experiments were performed from fall 1996 through spring 1999 to test the impact of *Meloidogyne incognita* on fruit yield of susceptible (Agriset 761, Florida 47) and nematode resistant (Sanibel) tomato cultivars in central Florida. After five continuous planting cycles, root gall severity, final harvest soil population density, and tomato yield losses were higher on Sanibel compared to susceptible Agriset or Florida 47. During spring 1999, the higher degree of plant tolerance and nematode resistance was restored to the original level of fall 1996 when Sanibel was planted into *M. incognita* infested microplots formerly cropped exclusively with susceptible Agriset or FL 47. Temperature controlled growth room studies (24 °C, 32 °C) confirmed the loss of resistance at high temperature and the development of a resistance breaking biotype of *M. incognita*.

DAMAGE AND REPRODUCTION OF TWO *PRATYLENCHUS VULNUS* ISOLATES ON *PRUNUS* ROOTSTOCKS IN THE SOUTHEASTERN UNITED STATES. **Nyczepir, A. P.,[1] and J. Pinochet.[2]** [1]Southeastern Fruit and Tree Nut Research Laboratory, ARS, USDA, Byron, GA 31008, and [2]Agromillora Catalana, S. A. El Rebato s/n, 08739 T. M. Subirats, Barcelona, Spain.

Guardian, Lovell, and Nemaguard peach rootstocks were evaluated for their susceptibility and growth response to two isolates of *Pratylenchus vulnus*. One nematode isolate was obtained from peach in Georgia (Pv-GA) and the other from apple in Idaho (Pv-ID). Nematode reproduction and pathogenicity as related to rootstock were determined 29 months after inoculation in outdoor microplots (25.4-cm-d × 30.5-cm-deep). Both nematode isolates reproduced on all rootstocks. A greater ($P < 0.05$) number of nematodes per gram dry root weight were recovered from Guardian as compared to Lovell or Nemaguard rootstocks. All rootstocks supported greater numbers of Pv-GA than Pv-ID. Tree growth as measured by fresh shoot and root weights and trunk diameters was greater in the uninoculated, intermediate in Pv-ID, and most suppressed in Pv-GA plots.

INFLUENCE OF SUSCEPTIBLE AND RESISTANT COWPEA PLANTS ON LIFE HISTORY TRAITS OF *MELOIDOGYNE INCOGNITA*. **Petrillo, M. D., and P. A. Roberts.** Department of Nematology, University of California, Riverside, CA 92521.

The root-knot nematode, *Meloidogyne incognita*, reproduces by mitotic parthenogenesis. Genetic information is passed from mother to progeny in the absence of sexual reproduction. Isofemale lines derived from an isolate are expected to be genetically homogeneous. Life history traits were tested in two populations isolated from the same field and in isofemale lines developed from these populations. Hatch and root penetration, reproduction, and fecundity on the susceptible host were highly variable among isolates. Hatch ranged from 0.4 to 7.3% and root penetration ranged from 3.1 to 44.4%. Mean egg-mass numbers per root system ranged from 3.7 to 21.1% of a 1,000 J2 inoculum and mean eggs per egg-mass ranged 360 to 665. Isolates could be grouped according to fitness levels that resulted from successive culturing on susceptible or resistant cowpea genotypes. Continuous selection on plants with or without the *Rk* gene and initial ability to overcome resistance conferred by *Rk* were the most significant factors determining isolate persistence. Isolates became extinct or shifted in virulence and fitness over 16 generations. These results suggest that *M. incognita* populations are a heterogeneous group of individuals varying in fitness, virulence to the Rk, gene and inheritance of genetic information from generation to generation.

THE UTILITY A COMPUTER PROGRAM FOR THE INTEGRATED CONTROL OF POTATO CYST NEMATODES. **Phillips, M. S.,[1] M. Elliott,[1] J. W. McNicol,[2] and D. L. Trudgill.[1]** [1]Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA, UK; and [2]Biomathematics and Statistics Scotland, SCRI, Dundee, DD2 5DA, UK.

A computer-based program for modeling nematode population dynamics and yield losses was developed using data from field trials at different sites prepared with plots spanning a range of population densities of potato cyst nematode (PCN, *Globodera pallida*). Potato yields and PCN multiplication were well-described by density dependent equations which are potentially predictive by incorporating functions for consistent site and cultivar differences in tolerance. The current program allows for differences in site and cultivar tolerance, yield potential and resistance. Different management strategies can be examined by changing the effectiveness of nematicides and(or) resistant cultivars as well as the duration and rates of PCN decline during the years between potato crops. The program has demonstrated the difficulties many farmers face in managing *G. pallida*.

CONSUMER PERSPECTIVES ON FOOD BIOTECHNOLOGY. **Pitman, Susan.** International Food Information Council, 1100 Connecticut Avenue, N.W., Suite 340, Washington, DC 20036.

Consumer acceptance is crucial to the future of food biotechnology, but consumer views vary dramatically from country to country. The International Food Information Council has been working since the early 1990s to inform the public on food biotechnology. As part of this effort, extensive quantitative and qualitative research has been conducted to determine how consumers—particularly U.S. consumers—truly feel about food biotechnology. Results from IFIC's consumer attitudinal research on food biotechnology conducted from 1996 through 1999 will be summarized. Consumer research results reveal opportunities and barriers to address when communicating about food biotechnology and indicate that the general public is accepting of, and would like to learn more about, these issues. In addition, as we consider the impact of labeling and education on consumer acceptance of foods produced using agricultural biotechnology, the critical importance of language is often overlooked. IFIC's consumer research results are compared to other research to provide insights on how language affects consumer acceptance of food biotechnology. Scientific jargon, although accurate, can invoke negative reactions from consumers unfamiliar with biotechnology.

CRYOPRESERVATION OF A MERMITHID NEMATODE. **Platzer, E. G., and C. L. Legaz**. Department of Nematology, University of California, Riverside, CA 92521.

Dimethylsulfoxide (DMSO) at a concentration of 2.5% has proven to be very effective as a cryoprotectant. The infective juveniles of *Romanomermis culicivorax* after exposure to DMSO and freezing in liquid nitrogen swim actively and appeared viable. In our first trial with nematodes treated with levamisole and cryoprotected with DMSO, 3.5% of the nematodes were infective for mosquitoes. In a subsequent trial, the nematodes appeared viable but were not infective for mosquito larvae. We surveyed the potential anesthetic effects of 13 additional potential inhibitors of nematode motility and found two calcium channel blockers, verapmil and diltiozem, that effectively blocked motility. In addition, sodium azide was effective as a reversible inhibitor of nematode motility. However, although sodium azide in the presence of DMSO was effective in preserving nematode motility post cryopreservation, infectivity was not protected.

GREENHOUSE STUDIES ON THE EFFECTS OF SOIL HEATING AND INCORPORATION OF BRASSICA PLANT WASTE ON CONTROL OF *MELOIDOGYNE INCOGNITA* ON MELON. **Ploeg, A. T.** Department of Nematology, University of California, Riverside, CA 92521.

Plastic vials (250 ml) were filled with *M. incognita*-infested sand. Treatments consisted of adding or not adding to sand finely chopped fresh Broccoli leaf and stem material (2 g/100 g 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. The lids were removed, and the vials were left on a greenhouse bench for 1 day. A melon seed (var. Durango) was then planted in each vial and the melon seedling was grown for 4 weeks. Each treatment combination had 5 replicates. After 4 weeks the melon plants were washed from the vials and root and top fresh weight, root galling, and number of egg masses was recorded. Data were analyzed using ANOVA procedures. Results showed that at temperatures above 25 °C, incorporation of broccoli plant waste strongly reduced galling of melons. The effect of incorporation of broccoli on root galling of melon occurred sooner at higher temperatures. Phytotoxicity due to soil heating or incorporation of broccoli was not observed. The potential for using a combination of soil heating and incorporation of broccoli plant waste to control *M. incognita* will be evaluated in field studies.

THE DAMAGE FUNCTION AND PF/PI RELATIONSHIPS OF *MELOIDOGYNE INCOGNITA* ON MELON *(CUCUMIS MELO).* **Ploeg, Antoon T.,[1] and M. S. Phillips.[2]** [1]Department of Nematology, University of California, Riverside, CA 92521, and [2]Department of Nematology, Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA, Scotland.

Melons grown in pots were inoculated with a range (0–1500 J2/100 g soil) of *M. incognita.* Plants were inoculated at seeding or 2 or 4 weeks after seeding. Eight weeks after inoculation, plant growth and nematode infestation data were collected. Data were fitted according to Seinhorst's yield loss model. Damage to plants inoculated at seeding was severe, with no plants surviving inoculum levels over 188 J2/100g soil. However, plants inoculated 2 or 4 weeks after seeding all survived even at the highest inoculum level. Differences in nematode tolerance limits were not significant between the 0, 2 or 4 week-old plants. The estimated minimum yield however was significantly higher when plants were 2 or 4 weeks old at time of inoculation. Increasing inoculum densities caused severe root galling, an increase in root weight and in the percentage dry matter of the tops. Analysis of final root population levels showed that the age of the melons at time of inoculation did not affect the nematode maximum reproduction rate, but that estimated maximum population levels increased when older plants were inoculated. A related study on an *M. incognita*-infested field site showed that fruit yields (kg/plant) started to decrease at very low nematode densities. Yield losses of approximately 65% were estimated at the highest nematode densities. The number of fruits/plant was more strongly affected than the fruit size.

A RELATIONSHIP BETWEEN PLANT SPECIES DIVERSITY, SOIL NEMATODE DIVERSITY, AND ECOSYSTEM PROCESSES. **Porazinska, D. L.,[1] A. N. Parsons,[1] H. W. Hunt,[1] T. R. Seastedt,[2] and D. H. Wall.[1]** [1]Natural Resource Ecology Laboratory, Colorado State University,

Fort Collins, CO 80523-1499 and [2]Environmental Population and Organismal Biology, University of Colorado, Boulder, CO 80309-0450.

We hypothesized that the diversity of soil microbial and faunal assemblages depends on the diversity and resource quality of plant communities. Furthermore, the rate of ecosystem processes (e.g. nutrient mineralization) is affected by the diversity of soil biota. To determine whether plants influence the diversity of belowground communities, we assessed nematode community structure (trophic level) under scenarios of various plant species and plant resource quality (C3 vs C4) combinations at the Konza LTER (tall-grass prairie). Only plant parasitic nematodes were affected by plant species composition. While nematode communities (abundance of trophic groups and trophic group diversity) did not respond to plant resource quality and resource diversity, they were influenced by the richness of plant species. Omnivorous nematodes and overall diversity of nematode communities were significantly higher under heterogeneous than homogenous plant communities. Plant species richness significantly affected C, but not N mineralization rate. Our results indicate that in this ecosystem soil biota diversity and C mineralization depend more on the richness of plant species than on resource quality.

SUSCEPTIBILITY OF APHID MIDGES, *APHIDOLETES APHIDIMYZA* (DIPTERA: CECIDOMYIIDAE), TO ENTOMOPATHOGENIC NEMATODE INFECTION. **Powell, J. R.,[1] and J. M. Webster.[1]** [1]Department of Biological Sciences, Simon Fraser University, Burnaby, BC V5A 1S6.

Entomopathogenic nematodes (EPNs) of the genera *Heterorhabditis* and *Steinernema* have a wide host range under experimental conditions, infecting hundreds of species in several insect orders, as well as some non-insect arthropod species. EPNs are used to manage several agricultural insect pests, and ecological and physiological barriers minimize or prevent their infection of non-target insects. It is generally accepted that inundatory applications of EPNs have no significant impact on non-target arthropod populations, but this assumption has not been rigorously tested. Aphid midges, *Aphidoletes aphidimyza* (Diptera: Cecidomyiidae), have soil-associated stages in their life cycles where EPN contact could occur. Inundatory releases of *A. aphidimyza* are used for greenhouse aphid management and naturally occurring populations reduce aphid numbers on cultivated plants. If susceptible to EPN infection, *A. aphidimyza* populations may be diminished such that aphid management is ineffective. Experiments are described that will determine if *A. aphidimyza* is susceptible to EPN infection, what life stages are susceptible and under what conditions.

NEMATODES FROM THE SANDHILLS FENS OF NEBRASKA. **Powers, T. O., A. L. Szalanski, and P. Mullin.** Department of Plant Pathology, University of Nebraska-Lincoln, Lincoln, Nebraska 68583.

The Sandhills of Nebraska are the largest sand-dune area in the Western Hemisphere. Underlying the Sandhills is the Ogalala aquifer, which feeds numerous wetlands in the region. Jumbo Valley Fen, located in Cherry Co. is a peat wetland the origin of which predates the sand-dune formation. Pollen analysis and radiocarbon dating of the basal peat layer (12,260 years B.P.) suggests that habitat was typical of a late Pleistocene spruce forest. This time coincides with the receding of the glacial ice sheet and a northern advance of boreal forest. Core samples from a transect across Jumbo Valley Fen contain active nematodes only in the upper 15 cm. Below that depth nematodes are present, however they appear preserved, reminiscent of organisms recovered from cool climate bogs in Europe. Two nematode species recovered from depths over one meter, *Hirschmanniella gracilis* and *Aorolaimus* sp., are remarkably well preserved and not found in the upper, active layer. These species may reflect changes in the fen vegetation during the last thousand years.

FATTY ACID BINDING PROTEINS OF PLANT PARASITIC NEMATODES. **Prior, Alison E.,[1] J. T. Jones,[1] V. C. Blok,[1] and M. W. Kennedy.[2]** [1]Unit of Mycology, Bacteriology and

Nematology, Scottish Crop Research Institute, Invergowrie, Dundee, DD2 5DA, UK, and [2]Division of Infection and Immunity, IBLS, University of Glasgow, Glasgow, G12 8QQ, UK.

Parasitic nematodes produce several families of fatty acid binding proteins. These proteins differ from similar proteins found in other animals because they are often secreted. The localization of these proteins on the parasite surface suggests they may have a role in the host-parasite interaction or in acquiring complex lipids which the parasites are unable to synthesize *de novo*. We have used a range of biochemical and biophysical techniques to investigate the function of a member of the nematode-specific LBP-20 family of proteins from *Globodera pallida*. The range of fatty acids (and other ligands) which bind to the *G. pallida* protein has been investigated and binding affinities for several ligands have been determined. Site-directed mutagenesis has allowed the identification of two residues that may play a role in ligand binding to the protein. We are also currently investigating potential new roles in lipid transport for nematode fatty acid binding proteins.

AN EFFICIENT cDNA-AFLP-BASED STRATEGY FOR THE IDENTIFICATION OF PUTATIVE PATHOGENICITY FACTORS FROM THE POTATO CYST NEMATODE *GLOBODERA ROSTOCHIENSIS.* **Qin, Ling,[1] H. Overmars,[1] G. Smant,[1] J. R. van der Voort,[1, 3] P. van Koert,[1] A. Schots,[2] J. Bakker,[1] J. Helder.[1]** [1]The Graduate School for Experimental Plant Sciences and [2]Laboratory of Monoclonal Antibodies, Wageningen University and Research Center, Binnenhaven 10, 6709 PD Wageningen, The Netherlands, and [3]Keygene N. V., Agro Business Park 90, P.O. Box 216, 6700 AE Wageningen, The Netherlands.

A new strategy has been designed to identify putative pathogenicity factors from the dorsal or subventral esophageal glands of the potato cyst nematode *Globodera rostochiensis*. Three independent criteria were used for selection. First, genes of interest should predominantly be expressed in infective second stage juveniles, with little or no expression in younger developmental stages. To screen for putative pathogenicity factors, gene expression profiles from five different developmental stages were generated using cDNA-AFLP. Second, the mRNA corresponding to such a putative pathogenicity factor should predominantly be present in the esophageal glands of preparasitic juveniles. Candidate mRNAs were tested for spatial localization *in situ* hybridization. As a third criterion, these proteinaceous factors should be preceded by a signal peptide for secretion. Expression profiles of more than 4,000 genes were generated and three up-regulated, dorsal gland-specific proteins preceded by signal peptide for secretion were identified. These represent the first genes expressed specifically in dorsal glands cloned from plant-parasitic nematodes. The partial sequence of these three factors, A4, A18 and A41, showed no significant homology to any known gene. Their presence in the dorsal glands of infective juveniles suggests that these proteins are involved in feeding cell initiation, and not in migration in the plant root or in protection against plant defense responses. Our results underscore the power of cDNA-AFLP as a reliable, rapid and efficient method to systematically identify differentially-expressed genes and can be used successfully in the studies of plant-pathogen interactions.

EFFICACY OF 1,3-DICHLOROPROPENE IN COMPOSTED AND NONCOMPOSTED SOIL. **Riegel, C.,[1] S. D. Nelson,[2] D. W. Dickson,[1] and L. H. Allen.[2]** [1]Entomology and Nematology Dept., and [2]USDA-ARS, Univ. of Florida, Gainesville, FL 32611.

When methyl bromide is phased out as a chemical control agent, 1,3-dichloropropene (1,3-D) is a likely alternative. Our objective was to determine root-knot nematode survival at three depths in two soil types after exposure to 1,3-D for various periods of time. The study was conducted twice in microplots containing composted or noncomposted soil infested with *Meloidogyne incognita*. 1,3-D was broadcast at 112 liters/ha. Controls were untreated plots of the two soils. One week after fumigation, six tomato 'Rutgers' seedlings were transplanted into each microplot and root galling was indexed 7 weeks later. Soil was collected from each microplot at 6, 24, 48, 72, and 96 hours after fumigation at three depths (0–15, 15–30, and 30–45 cm). Tomato 'Rutgers' was used to bioassay these samples for the number of viable J2 7 weeks after transplanting. Plants grown in

fumigated noncomposted soil had fewer galls than plants from fumigated composted soil ($P < 0.1$). Gall indicies from roots in fumigated composted soil were not different from the controls. From the soil bioassay, the number of galls decreased with increasing exposure in both soils at 0 to 15 and 15 to 30-cm-deep ($P < 0.05$). The number of galls at the lowest depth was not decreased likely because of higher soil water content.

MANAGING SOYBEAN CYST NEMATODE, *HETERODERA GLYCINES*, IN ONTARIO USING GREEN MANURE. **Riga, E., T. Welacky, E. Topp, J. Potter and A. Tenuta.** Agriculture & Agri-Food Canada, SCPFRC, Vineland St., Ontario, L0R 2E0 Canada.

The soybean cyst nematode, *Heterodera glycines*, is the most economically important pest of soybeans (*Glycine max*) in Ontario and the need to control it is great. The potential of green manure and plant root exudates from a range of plant species to protect soybean against *H. glycines* was examined in vitro and under greenhouse conditions. Green manure from *Lespedeza capitata, L. Intermedia, L. hirta, Lolium multiflorum, L. perenne, Lupinus perennis, Melilotus officinalis, Medicago sativa, Trifolium pratense*, Fairway ''B'' Lawngrass mixture and *Pisum sativum* reduced the number of *H. glycines* on soybean roots and in the soil around the plants. Root exudates of *L. capitata, Trifolium hybridum, T. repens, L. multiflorum, L. perennis, M. sativa* and *G. max* increased the egg hatching rate of *H. glycines* in comparison to the water control. Root exudates of *T. hybridum, T. repens* and *L. multiflorum* caused elevated hatching of *H. glycines* eggs in comparison to the root exudates of soybeans. In addition, root exudates of *T. repens, L. multiflorum, Echinochloa crusgalli, L. perennis, T. hybridum, M. sativa* and soybean increased neutral lipid utilization of *H. glycines* juveniles in comparison to the control. From the plant species tested, *L. multiflorum* is the most effective for depleting populations of *H. glycines.*

PRATYLENCHUS AND *HETERODERA* IN CROPPING SOILS OF WESTERN AUSTRALIA. **Riley, I. T.,[1] and S. J. Kelly.[2]** [1]Applied and Molecular Ecology, University of Adelaide, Glen Osmond SA 5064, and [2]Agriculture Western Australia, South Perth WA 6151, Australia.

A systematic survey of *Pratylenchus* and *Heterodera* centered on the 40 shires with the highest proportion of cereal cropping. About 400 soil samples were collected in 1997 and 1998 at 10-km-intervals along N-S transects 35 km apart. A targeted survey in 1997 examined about 100 soil samples from fields with unexplained poor crop performance. *P. neglectus* was most commonly detected, followed by *P. thornei* and *P. zeae.* Populations of *P. brachyurus, P. penetrans, P. scribneri*, and an undescribed species were also found. *Pratylenchus* was found in 64% of systematically collected samples and 60% of the targeted samples. The distribution was non-uniform with some areas having relatively light infestations. Potentially damaging *Pratylenchus* populations occurred in a significant proportion of fields. *Heterodera* was not found in any survey sample. Further examination of the factors contributing to spatial variation in *Pratylenchus* populations may reveal suitable approaches for management, potential for biocontrol and (or) factors that predict the risk of crop damage.

RENIFORM NEMATODE RESISTANCE IN SOYBEAN, 1999 TESTS. **Robbins, R. T.,[1] L. Rakes,[1] L. E. Jackson,[1] and D. G. Dombek.[2]** [1]Plant Pathology Department, and [2]Arkansas Crop Improvement Program, University of Arkansas, Fayetteville, AR 72701, USA.

Two hundred twenty-six soybean cultivars were tested in the greenhouse to identify resistance to the reniform nematode (*Rotylenchulus reniformis*). The cultivars tested were new entries into the Arkansas and Mississippi soybean testing programs and entries submitted by extension nematologists from Auburn University and Louisiana State University. Included in the tests as controls were the resistant cultivars Forrest and Hartwig, the susceptible check Braxton, and fallow infested soil. Five replications of each test treatment were inoculated with 3,450 eggs and vermiform reniform nematodes and grown for 10 weeks in 10 cm-diam. pots. Numbers of reniform nematode extracted from the soil and roots and the ratio of the number reproducing on Forrest were calcu-

lated. Cultivars with reproduction not different from Forrest ($P > 0.05$) were termed resistant, whereas those with greater reproduction than Forrest were termed susceptible. One of 12 cultivars in the relative maturity group (RMG) 4.4 or less was termed resistant, 24 of 72 cultivars of RMG 4.5 to 4.9 were resistant, 9 of 41 cultivars of RMG 5.0 to 5.4 were resistant, 11 of 66 cultivars of RMG 5.5 to 5.9 were resistant, and 10 of 35 cultivars of RMG 6.0 or greater were resistant.

ANALYSIS OF PLANT PROMOTERS INDUCED BY CYST AND ROOT-KNOT NEMATODES. **Robertson, Lee,[1] C. Ilett,[1] S. Sanz-Alférez,[1] R. Estévez,[1] and C. Fenoll.[1, 2]** [1]Departamento de Biología, Universidad Autónoma de Madrid, E-28049Madrid, and [2]Facultad de CC del Medio Ambiente, Universidad de Castilla-La Mancha, E-45071 Toledo, Spain.

Cyst-forming and root-knot nematodes are interesting from biological and economic perspectives. Second stage juveniles of these nematodes invade roots and establish complex nematode feeding sites (NFS). This process involves morphological and physiological changes, accompanied by altered gene expression in the plant, suggesting the nematodes manipulate fundamental plant processes. Previous studies have described the induction of specific plant mRNAs and activation of various plant promoters at the NFS. The goal of our laboratory is to understand these changes in the transcriptional machinery of the cell and the differentiation processes within the developing NFS. The availability of differentially regulated promoters has made it possible to investigate these changes. We will present our work on the characterization of sequences in various promoters, which may be related to their nematode responsiveness. Functional analysis is being used to identify nematode responsive elements. These studies may aid the design of specific nematode-inducible promoters for transgenic nematode resistance. The study of the signal cascades initiated by nematodes and the search for feeding site-specific transcription factors will improve our understanding of NFS development.

STATE OF KNOWLEDGE REGARDING SOURCES OF RESISTANCE TO THE RENIFORM NEMATODE (*ROTYLENCHULUS RENIFORMIS*) IN UPLAND COTTON (*GOSSYPIUM HIRSUTUM*). **Robinson, A. F., and A. E. Percival.** USDA, ARS, SPARC, College Station, TX 77845.

Within the last 10 years, the reniform nematode (*Rotylenchulus reniformis*) has become recognized as the most serious nematode problem of cotton in Louisiana, Mississippi, and Alabama. The Cooperative Extension Service in each state has focused on nematicide and crop rotation studies to provide farmers with management options, which in most cases are marginally cost effective, because no known cultivar and only four breeding lines have been reported to show any resistance whatsoever to this nematode. Previous studies cumulatively examining about 250 genotypes indicated that varying levels of resistance may reside in primitive accessions of *G. hirsutum* and related species. In recent growth chamber experiments, we have directly compared all previously reported resistant *Gossypium* accessions for which seed is available, and in the greenhouse are currently testing 2,800 additional, previously unexamined primitive accessions of *G. hirsutum* and the closely related *G. barbadense*. Results so far include: confirmation of immunity in *G. longicalyx*, high resistance in several accessions of *G. arboreum* and *G. herbaceum*, moderate resistance in three accessions of *G. barbadense* (USDA accessions TX 110, 1347, and 1348), and weak resistance in four breeding lines of *G. hirsutum*. Most accessions of *G. hirsutum* and *G. barbadense* support high levels of reproduction comparable to contemporary cultivars.

ANALYSIS OF STYLET ACTIVATION USING ELECTRO-PHARYNGEOGRAM TECHNIQUES. **Rolfe, R. N., and R. N. Perry.** Entomology and Nematology Department, IACR-Rothamsted, Harpenden, Herts AL5 2JQ, UK.

The electropharyngeogram (EPG) technique was first used to analyse pharyngeal pumping of *Caenorhabditis elegans.* We have used the EPG technique to record electrical activity of stylet protractor muscles of fourth-stage juveniles of *Ditylenchus dipsaci* and second-stage juveniles of *Globodera rostochiensis*. Individual nematodes were captured by the anterior end in a suction

pipette electrode, from which the electrical activity was recorded. Stylet activity was initiated by exposure to various concentrations of the neurotransmitter serotonin (5-hydroxytryptamine), and the changes in membrane electrical potential corresponding to the excitatory and repolarisation phases of the stylet protractor muscles were recorded. The responses to selected agonists and antagonists have been analysed to provide information on nematode neurotransmission and to illustrate the potential of this technique to evaluate stylet activity.

DISCOVERY OF A SYNERGY BETWEEN SPATIALLY SEPARATED *PRATYLENCHUS PENETRANS* AND *VERTICILLIUM DAHLIAE* IN RUSSET BURBANK POTATO RESULTING IN IMPAIRED PHOTOSYNTHESIS. **Rotenberg, D., and A. E. MacGuidwin.** Dept. Plant Pathology, University of Wisconsin, Madison, 53706.

Pratylenchus penetrans (Pp) interacts synergistically with *Verticillium dahliae* (Vd) to cause potato early dying (PED) when the two pathogens infect potato roots at low densities. Using an artificial method of fungal inoculation, we conducted growth chamber experiments to determine if synergy, as measured by reduced light use efficiency (LUE), exists when the two organisms occupy separate plant organs of Russet Burbank potato. We achieved pathogen separation by inoculating potato roots with Pp 3 days prior to injecting Vd conidia into the stem vasculature. Treatments were Pp alone, Vd alone, Pp and Vd together, and a non-inoculated control. Gas exchange was measured nondestructively in the fifth youngest leaf at 2-day intervals after stem injection with Vd. Stems were harvested 16 days post-injection and Vd colony forming units (CFU) were extracted from stem sap. Joint infection reduced

($P = 0.01$) LUE during the course of the experiment, while single-pathogen inoculations had no impact. Synergy occurred 6 days post-injection with the fungus and remained after 12 days. The nematode enhanced ($P = 0.087$) the number of CFU of the fungus in potato stems. Without discounting interactions between the nematode and fungus during early infection events in potato roots, our results indicate that Pp and Vd interact synergistically to impair photosynthesis when they occupy separate potato tissues.

MOLECULAR ANALYSIS OF RIBOSOMAL DNA SEQUENCE DATA FOR FOUR SPECIES OF CYST NEMATODES FROM ITALY AND ONE FROM SYRIA. **Sabo, A.,[1] N. Vovlas,[2] and V. R. Ferris.[1]** [1]Department of Entomology, Purdue University, West Lafayette, IN 47907-1158, and [2]Istituto di Nematologia Agraria, C.N.R. Via Amendola 165/A, 71426 Bari, Italy.

Phylogenetic analysis of new ribosomal DNA (rDNA) data for *Heterodera mediterranea, H. hordecalis, H. carotae* and *H. fici* from Italy; and *H. ciceri* from Syria, along with published data for other species, showed >95% bootstrap support for the following relationships: (((((*H. carotae, H. cruciferae*), *H. goettingiana*), (((*H. trifolii, H. ciceri*), *H. mediterranea*), ((*H. avenae, H. latipons*), *H. fici*))), (*Cactodera betulae, H. hordecalis*)), (*Globodera rostochiensis, G. pallida*)). The rDNA sequence data were for the two internal transcribed spacers (ITS1 and ITS2) plus the 5.8S gene between them. These inferred relationships support the classic ''*Goettingiana* Group'' of *H. carotae*, *H. cruciferae*, and *H. goettingiana*. A clade comprised of *Cactodera betulae* and *H. hordecalis* is only distantly related to the other species in the analysis.

DETERMINATION OF PLANT-PARASITIC NEMATODES ASSOCIATED WITH KIKUYU-GRASS (*PENNISETUM CLANDESTINUM*) IN COSTA RICA. **Salazar, L., and M. Quesada.** CIPROC, University of Costa Rica, Costa Rica.

Fifteen genera are reported in association with Kikuyugrass in Costa Rica. The survey was undertaken in the years 1998 to1999 in 16 localities dedicated to dairy production. A total of 181 composited soil and root samples were taken. The most frequent genera found in soil were *Tylenchus* spp., *Helicotylenchus* spp., *Criconemella* spp., *Heterodera* sp., *Trichodorus* spp., *Xiphinema* spp., *Boleodorus* sp., *Meloidogyne* spp., *Hemicycliophora* sp., and *Pratylenchus* spp. The most frequent genera found in roots were: *Tylenchus* spp., *Helicotylenchus* spp., *Pratylenchus* spp.,

Heterodera spp., *Meloidogyne* spp. and *Ditylenchus* spp. Other genera that were found less frequently in soil were *Criconema* spp., *Paratylenchus* spp., *Lobocriconema* spp. and *Ditylenchus* spp., and in roots were *Aphelenchoides* spp., *Hemicycliophora* spp., *Boleodorus* spp. and *Scutellonema* spp. *Pratylenchus* spp., *Tylenchus* spp., *and Ditylenchus* spp. *Helicotylenchus* spp. and *Meloidogyne* spp. showed the highest mean populations meanwhile *Pratylenchus* spp. showed the highest density of population.

RESISTANCE OF JAPANESE SWEET POTATO CULTIVARS TO TWO ISOLATES OF *MELOIDOGYNE INCOGNITA*. **Sano, Z.[1] H. Iwahori,[1] and E. Kawano.[2]** [1]Kyushu National Agricultural Experiment Station, Nishigoshi, Kumamoto 861-1192, and [2]Faculty of Agriculture, University of Miyazaki, Miyazaki 889-2192 Japan.

Meloidogyne incognita isolates Ns and Mz were collected in Kyushu, Japan, and reared from single egg masses. Cuttings of 23 major sweet potato cultivars inoculated with newly hatched J2 were grown in a green house at an average temperature of 27 °C and reproduction rates (Rr; eggs produced per J2 inoculated) were calculated after 35 days of growth. In three cultivars with different resistance, rate of penetration and histological reactions of root tissues were examined. On 9 cultivars Rr were lower than 1 for both isolates. On four other cultivars, distinct differences were observed between Rr of Ns and Mz. Both isolates reproduced well on the remaining 10 cultivars. J2 of Ns penetrated roots of all three cultivars equally well but in the highly resistant cv. Norin No. 2, there was a hypersensitive reaction (HR) after J2 penetration and very few of the J2 in roots became enlarged. In the moderately resistant cv. *Beniazuma*, the HR was not conspicuous but the number of egg masses was 25% of the susceptible cv. Norin No. 1.

CONTROL OF CORKY RINGSPOT DISEASE ON POTATO IN EASTERN WASHINGTON. **Santo, G. S., and J. H. Wilson.** Department of Plant Pathology, Washington State University, Prosser, WA 99350.

Corky ringspot disease (CRS), which is caused by the tobacco rattle virus (TRV), and vectored by *Paratrichodorus allius*, is a serious problem in potato production in the Pacific Northwest. Nematicide trials were conducted in 1996, 1997 and 1999 on Norkotah potato in a commercial field near Pasco, WA infested with *P. allius* carrying the severe strain of TRV. Treatments included 1,3-dichloropropene (1,3-D) shank-injected 46 cm at 94, 140, and 187 liters/ha, metam sodium (MS) at 351 liters/ha shank-sprayed (SS) 15 cm with sweep shanks attached with spray nozzles, MS 230 liters (SS, 36 cm) + 153 liters applied as a surface broadcast and incorporated 15 cm deep (SB), 1,3-D 94 liters + MS 280 liters (SB), 1,3-D 140 liters + MS 187 liters (SB) and MS 280 liters (SB), 1,3-D 187 liters + aldicarb 15G 3.24 kg/ha at plant (AP) and post plant (PP), MS 230 liters (SS, 36 cm) + 153 L (SB) + aldicarb 3.24 kg, ethoprop 6EC 13 kg a.i./ha + aldicarb 3.24 kg, and aldicarb 3.24 kg AP and PP. Results showed 1,3-D at 140 and 187 liters/ha alone or in combination with other treatments, 1,3-D 94 liters/ha in combination with MS, and aldicarb PP gave excellent control of CRS. None of the other treatments provided adequate control.

BIOLOGICAL CONTROL OF SOD WEBWORM LARVAE (LEPIDOPTERA: PYRALIDAE) WITH ENTOMOPATHOGENIC NEMATODES IN QUEBEC. **Simard, Louis,[1] J. Brodeur,[1] and G. Bélair.[2]** [1]Centre de recherche en horticulture, Université Laval, Québec, Canada G1K 7P4, and [2]Agriculture and Agri-Food Canada, St-Jean-sur-Richelieu, Quebec Canada J3B 3E6.

In summer of 1999, the virulence of four entomopathogenic nematode species was tested against the sod webworm larvae (*Chrysoteuchia topiaria*), a turfgrass pest in Quebec. In the laboratory, larvae were exposed to eight concentrations of each species in Petri dishes at 24 °C for a 5-day period. *Heterorhabditis megidis* was the most virulent species with a LD50 of 6 nematodes/larva. *Steinernema glaseri*, *S. carpocapsae*, and *S. feltiae* revealed LD50 values of 34, 68, and 126, respectively. The effect of nematode exposure time on *C. topiaria* larval mortality was evaluated with *H. megidis* and *S. carpocapsae*. With a concentration of 1,000 nematodes/larva, a 24-hour

exposure time or more was required to reach 80% mortality rates. Preliminary field tests against sod webworm larvae were conducted with *H. megidis, S. carpocapsae,* and *S. feltiae* on several residential lawns located in Quebec City and Montreal. In some lawns, sod webworm larval populations were substantially reduced by nematode treatments when compared to the untreated control plots. The efficiency of nematode applications was comparable with the Diazinon insecticide treatment on most sites. A contagious distribution of sod webworm populations was observed on all tested sites. An effective monitoring of this insect would permit targeted nematode applications and thus reduce the use of insecticide in urban areas.

AN EXPLORATION OF THE ROLE OF DAF-21 IN NEMATODE DEVELOPMENTAL ARREST AND ITS POTENTIAL AS A NEW PHYLOGENETIC CHARACTER. **Skantar, Andrea M., and L. K. Carta.** Nematology Lab, USDA-ARS Plant Sciences Institute, Beltsville, MD 20705.

The daf-21 gene is a component of a neurosensory biochemical pathway that controls the formation of arrested (dauer) larvae in *Caenorhabditis elegans*. Daf-21 encodes a protein in the heat shock (HSP90) protein family. Proteins of this class are expressed in response to stress, and some have been shown to act as molecular chaperones, protecting other proteins from unfolding. Mutations in HSP90 have been shown to unmask hidden genetic variation in fruit flies and may therefore play a critical role in regulating the evolutionary potential of an organism. We have developed a robust set of PCR primers and amplification conditions that allowed us to isolate genomic clones with significant homology to HSP90 from several plant-parasitic nematodes, including *Heterodera glycines, Meloidogyne javanica, M. arenaria, Bursaphelenchus xylophilus,* and several of the Pratylenchinae. Sequence analysis has shown that these genes are highly conserved with other family members, with as much as 57% identity at the nucleotide level; however, the introns in some nematode genes differ from *C. elegans* in both number and location. Isolation of full-length genomic and cDNA clones are underway. We are also investigating the usefulness of this HSP90 gene as a new molecular phylogenetic character.

IMPACT OF INUNDATIVE APPLICATION OF ENTOMOPATHOGENIC NEMATODES ON NON-TARGET NEMATODE COMMUNITIES IN TURFGRASS ECOSYSTEM. **Somasekhar, N., E. A. B. De Nardo, and P. S. Grewal.** Department of Entomology, OARDC, Ohio State University, Wooster, OH 44691.

Biological pest control using entomopathogenic nematodes has been thought to be ecologically safe and risk-free. However, there is little information on impact of these biological control agents on non-target soil organisms, especially the soil nematodes. We evaluated the response of nematode communities in turfgrass ecosystems to inundatory application of native (*Heterorhabditis bacteriophora* strains HP88 and GPS11) and exotic (*H. indica*) species of entomopathogenic nematodes in comparison to application of a chemical insecticide, Trichlorfon (Dylox 80). Application of both native and exotic species of entomopathogenic nematodes resulted in a decrease in the population of plant-parasitic nematodes but the population of free-living nematodes was unaffected. In contrast, the application of chemical insecticide significantly decreased the population of both plant-parasitic and free-living nematodes with greater reduction in the population of free-living nematodes. These results show the beneficial (non-target) effect of the application of entomopathogenic nematodes in turfgrass. The possible reasons for the differential response of soil nematode communities to application of entomopathogenic nematodes (native and exotic species) and chemical insecticides are discussed.

SCREENING RHIZOBACTERIA FOR ROOT GROWTH PROMOTION AND REDUCTION OF MELOIDOGYNE HAPLA DAMAGE ON CARROT. **Soufiane, Brahim,[1] G. Bélair,[1] and C. Beauchamp.[2]** [1]Agriculture and Agri-Food Canada, Saint-Jean-sur-Richelieu, Quebec, Canada J3B 3E6, and [2]Université Laval, Dépt. Phytologie, Ste-Foy, Quebec, Canada G1K 7P4.

One hundred and sixty rhizobacteria isolates were recovered from a root-knot infested carrot field with organic soil and tested for their ability to promote carrot growth and to reduce damage caused by the northern root-knot nematode, *Meloidogyne hapla*. All isolates were screened for root growth promotion using alginate-coated carrot seeds in Petri dishes. Twelve isolates promoted root growth and were identified in greenhouse tests. Carrot seeds were coated and sowed in both pasteurised organic and sandy soil without nematodes. After a 2-month growth period, isolate 2F15 increased root length as well as stem and secondary root weights in organic soil but had no effect on treated carrots grown in sandy soil. Isolate 3B15 increased carrot root length, weight and size in mineral soil. Coated carrot seeds were sowed in pasteurized sandy soil inoculated with 1,200 second-stage juveniles per plant. Six rhizobacteria stimulated carrot growth and decreased nematode damage. Isolates 3B15 and 2F15 reduced respectively *M. hapla* induced symptoms by 20 and 10 %, respectively, whereas all control carrots were damaged. Galling on secondary roots was not reduced by any of the isolates and no rhizobacteria was shown to be pathogenic to *M. hapla*.

EFFECT OF OZONATED AND UNOZONATED SWINE MANURE AND URINE ON HATCHING AND SURVIVAL OF *HETERODERA GLYCINES*. **Stein, Carolyn,[1] S. J. Masten,[2] H. Melakeberhan,[1] and M. T. Yokoyama.[3]** Departments of [1]Entomology, [2]Civil and Environmental Engineering, and [3]Animal Science, Michigan State University, East Lansing, MI, 48824.

The effect of ozonated and unozonated swine manure slurry and urine, zinc sulfate, and distilled water (controls) on the hatching and survival of *Heterodera glycines* was studied in two laboratory experiments conducted at 27 °C. Roughly 300 eggs were added to 3.7 ml of the respective treatment solutions and incubated in covered plastic dishes for 21 days. To test the effect of odor on hatching and survival, one set of controls was incubated apart from the other treatments. Hatching and mobility were microscopically observed and recorded every other day. Approximately 20% hatched in the unozonated swine manure slurry, and 40% hatched in the ozonated swine manure and urine, but none survived. About 80% of the eggs hatched in the controls, and 20% to 30% of those incubated separately survived at about 10 days. The results indicate that *H. glycines* is more sensitive to unozonated swine manure slurry than to the ozonated treatments. Further tests need to be done at lower rates than used in this study.

THE GENERA *PANAGROBELUS* (THORNE, 1939) AND *PLECTONCHUS* (FUCHS, 1930) (NEMATODA: PANAGROLAIMIDAE): MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION OF *PANAGROBELUS STAMMERI* (RÜHM, 1956) AND DESCRIPTION OF A NEW *PLECTONCHUS* SPECIES FROM THE UNITED KINGDOM. **S. P. Stock,[1*] I. De Ley,[2] M. Mundo,[3] S. Nadler,[1] and J. G. Baldwin.[3]** [1]Department of Nematology, University of California Davis, One Shields Ave., Davis, CA 95616-8668, [2]Instituut voor Dierkunde, Uni-versiteit Gent, Ledegankstraat 35, B-9000 Gent, Belgium, and [3]Department of Nematology, University of California Riverside. Riverside, CA 92521.

There is renewed interest in cephalobs of the genera *Panagrobelus* and *Plectonchus,* because they are currently the focus of molecular and developmental studies. However, one limitation to these investigations is inadequate understanding of the systematics and morphology of these genera. Efforts to obtain detailed morphological data by reexamination of types have been unsuccessful because the type specimens cannot be found. The absence of appropriate type specimens underscores the need for investigation of new isolates, such as the those represented by living specimens needed for molecular and developmental studies. In the present study, we have redescribed the genera *Panagrobelus* and *Plectonchus*, based on *Panagrobelus stammeri* Rühm and a new undescribed *Plectonchus* species. Isolates from England (PDL0024 and PDL0025, respectively) were used for detailed light and scanning electron microscope observations. Nucleotide sequence data (nuclear rDNA, D2/D3 28S) were obtained and analyzed to develop molecular diagnostic information and a phylogenetic context for these taxa. Additionally, this hypervariable

rDNA region can provide characters for comparisons of other *Panagrobelus* and *Plectonchus* isolates, including previously described species, novel morphotypes, and potentially, cryptic species.

GENETICALLY MODIFIED ORGANISMS: THE FUTURE AND THE SCIENTISTS' ROLE**.** **Stuckey, Richard E.** Council for Agricultural Science and Technology, Ames, IA 50014-3447.

The term genetically modified organism (GMO) refers to organisms, most frequently plants, that have been altered by adding one or a few genes through recombinant DNA techniques. Most molecular biologists claim this is simply a further refinement of genetic selection that has gone on for many years. The opposition claims interference with nature will produce drastic irreversible consequences. They also fear and oppose the food supply being controlled by a few multinational corporations. What are the consequences of continuation of developing and marketing GMOs? What are the consequences of discontinuation of research using genetic engineering? Why is the public so fearful of this technology? Who should take responsibility for a distrusting public—manufacturers, scientists, government, media? Will this technology survive and if so, what will it deliver in the future? What is the role of scientists? How is CAST addressing this issue? The questions presented above are those that will be discussed. A projected scenario for the next several years and the long term will be shared.

NEMATODES ASSOCIATED WITH CORN IN NEBRASKA. **Szalanski, Allen L., P. G. Mullin, and T. O. Powers.** Department of Plant Pathology, University of Nebraska-Lincoln, Lincoln, Nebraska 68583.

Nematodes representing freeliving, plant-parasitic, and predaceous forms were isolated from soil samples collected from corn fields in central and eastern Nebraska. Sample sites represented continuous and rotated corn, with or without irrigation. At least one hundred nematodes selected at random from 100 cm^3 soil samples were identified to genus from each sample. Number of genera averaged 18 among the soil samples. Dominant plant-parasitic/fungal feeders varied considerably among samples, and included *Helicotylenchus, Hoplolaimus, Tylenchorhynchus, Xiphinema* and *Filenchus.* The most common predaceous forms were *Aporcelaimellus* and *Labronema,* with *Eudorylaimus* and *Thornus* being the dominant omnivorous feeders. Species of *Acrobeles, Protorhabdits,* and *Mesorhabditis* were the most abundant bacterial feeding nematodes. Dominant taxa and nematode community structure differed considerably from adjacent tallgrass and mixed grass prairie sites.

INFECTION BEHAVIOR OF THE NEMATODE *PHASMARHABDITIS HERMAPHRODITA* TO THE GRAY GARDEN SLUG, *DEROCERAS RETICULATUM*. **Tan, L., and P. S. Grewal.** Department of Entomology, Ohio State University, OARDC, Wooster, OH 44691-4096.

Phasmarhabditis hermaphrodita (Rhabditida: Peloderinae) is a parasite of slugs in the families *Arionidae, Limacidae, Milacidae* (Gastropoda: Stylommatophora). The nematode has been previously shown to reproduce on several different bacteria and no specific bacterial symbiont has yet been identified. We studied the mode and routes of penetration of the nematodes into adult *D. reticulatum* slugs. As the nematodes can potentially reproduce on dead slugs, slug feces, and on soil bacteria, the role of the dauer stage in the infection process was examined. Slugs were exposed to juvenile, adult, and dauer juvenile nematodes, and their mortality was recorded daily up to 10 days. The dead slugs were dissected to confirm the penetration of the nematodes. Only dauer juveniles infected slugs, thus confirming the hypothesis that the dauers serve as infective juveniles in these nematodes. To study the routes of penetration, the slugs were exposed to the dauer juveniles in Petri dishes. After, 4, 8, 16, 24, 48, and 96h, 10 slugs were removed, washed, killed and fixed in boiling water. They were then dissected carefully to determine the presence of nematodes in different body parts. The nematodes were first detected in the mantle cavity of the slugs 8h after exposure and

most nematodes were found in the mantle cavity throughout the experiment. Therefore, we conclude that the mantle cavity is the main route of penetration of the nematodes.

PROTEINIC VARIATION IN *MELOIDOGYNE* SPP. AS SHOWN BY TWO-DIMENSIONAL ELECTROPHOREGRAM ANALYSIS: IDENTIFICATION OF TWO HOMOLOGOUS FATTY-ACID-BINDING PROTEINS DICRIMINATING THE TWO EUROPEAN QUARANTINE ROOT-KNOT NEMATODES, *M. CHITWOODI* AND *M. FALLA.* **Tastet, X. C., F. Val, M. Lesage, L. Renault, M. Bossis, and D. Mugniéry.** UMR INRA / ENSAR ''Biologie des Organismes et des Populations appliquée à la Protection des Plantes'' B.P. 35327, Domaine de la Motte, 35 653 Le Rheu Cedex, France.

Variability of protein patterns from adult females of four major *Meloidogyne* species (*M. incognita, M. javanica, M. arenaria* and *M. mayaguensis*) and the two European quarantine nematodes (*M. chitwoodi* and *M. fallax*) were achieved by two-dimensional gel electrophoresis. The protein profiles were compared using a computer-assisted analysis system in two separate experiments. Species- discriminative and shared proteins have been identified. The similarity index and the genetic distances between the different species studied were calculated on the basis of homologous polymorphic protein spots. Dendrograms were constructed according to the UPGMA method. Significance of the results from phenetic study was assessed by bootstrap analysis. Two major proteins, Mcf-A67 and Mcf-B66, have been identified and discriminated between the two quarantine nematodes. These protein markers have been partially sequenced after enzymatic digestion. The internal amino acid sequences exhibited similarities to members of a family of low molecular weight intracellular lipid-binding proteins. From these sequences, a Mcf-A67 synthetic peptide, coupled to the keyhole limpet hemocyanin, was used to produce rabbit polyclonal antibodies. The serum obtained reacted only with *M. chitwoodi* and *M. fallax* antigens on a dot-blots assay with a single female. The accuracy of the method and the implications for *Meloidogyne* quarantine species diagnostics are discussed.

SAFETY ASSESSMENT OF GENETICALLY MODIFIED CROPS IN FOODS. **Taylor, Steve L.** University of Nebraska-Lincoln, 143 Food Industry Complex, Lincoln, NE USA 68583-0919.

The assessment of the safety of genetically modified foods is based largely on the concept of substantial equivalence. This concept was first elaborated by the Organization for Economic Cooperation and Development (OECD) in 1993 and has been an important element in the safety assessment of genetically modified foods on a worldwide basis ever since. Substantial equivalence embodies the concept that if a new food or food component is found to be substantially equivalent to an existing one, it can be treated in the same manner with respect to safety. In other words, the food or food component can be concluded to be as safe as the conventional product. Establishment of substantial equivalence is not a safety assessment in itself, but a dynamic, analytical exercise in the assessment of the safety of a new food relative to an existing food. This comparison may be a simple task or be a very lengthy undertaking depending upon the amount of available knowledge and the nature of the product under consideration. The assessment should lead to one of three possibilities. The genetically modified food could be found substantially equivalent to the conventional counterpart. Or, the genetically modified food could be found substantially equivalent to the conventional counterpart except for certain defined differences. In that case, any safety assessment testing would be focused upon the defined differences. This is the situation that exists for many of the current products of agricultural biotechnology. In such cases, the differences that must be evaluated would include possible toxicity of the novel protein, possible allergenicity of the novel protein, and transfer of antibiotic resistance to intestinal microorganisms. Finally, the genetically modified food could be found not substantially equivalent to any conventional food. In that case, more extensive safety testing would be required and labeling of this type of food would also likely be necessary.

HEAT STABILITY OF RESISTANCE TO THE SOUTHERN ROOT-KNOT NEMATODE IN PEPPER GENOTYPES HETEROZYGOUS FOR THE *N* GENE. **Thies, J. A., and R. L. Fery.** U.S. Vegetable Laboratory, USDA, ARS, Charleston, SC 29414-5334.

Expression of the *N* gene which confers resistance to *Meloidogyne incognita* in pepper, *Capsicum annuum*, is modified at high temperatures (28 and 32 °C). However, expression of the *N* gene in the heterozygous condition (*Nn*) has not been documented at any temperature. Responses of two isogenic pepper cultivars (differing at the *N* resistance locus), and the F_1 of the cross and its reciprocal were compared at 24, 28, and 32 °C in a growth chamber experiment. Pepper lines used were: Charleston Belle (CB–*NN*), Keystone Resistant Giant #3 (KRG–*nn*), PX-249 (CB × KRG –*Nn*), and PX-249r (KRG × CB–*Nn*). KRG was susceptible at all 3 temperatures. Responses of CB, PX-249, and PX-249r were similar; these lines exhibited high resistance at 24 °C, but resistances were compromised at 28 °C and 32 °C. However, average root gall severity indices for the resistant lines were 68%, 50%, and 26% lower than for KRG at 24, 28, and 32 °C, respectively. These results suggest that the *NN* and *Nn* resistances are expressed similarly at high temperatures and hybrid cultivars (*Nn*) may be useful for managing *M. incognita* in hot climates.

EFFECTS OF 1,3-DICHLOROPROPENE AND SELECTED HERBICIDES ON *MELOIDOGYNE INCOGNITA* / NUTSEDGE INTERACTIONS. **Thomas, S. H.,[1] J. Schroeder,[1] and L. W. Murray.[2]** [1]Entomology, Plant Pathology and Weed Science Department, and [2]University Statistics Center, New Mexico State University, Las Cruces, NM 88003-8003.

The southern root-knot nematode (RKN) and yellow (*Cyperus esculentus* = YNS) and purple nutsedge (*C. rotundus* = PNS) are severe pests of chile pepper in New Mexico. Nutsedges host RKN and transmit the nematode from tubers to young chile plants. In 1997–1999 YNS and PNS tubers from RKN-infested and noninfested plants were fumigated with 1,3-D (56 liters/ha) and bioassayed with chile seedlings to determine the effects of the fumigant on RKN transmission. Results confirmed that 1,3-D has no effect on YNS or PNS tuber germination, but RKN appeared to enhance YNS tuber germination. Fumigation reduced the infection rate of chile by RKN from associated PNS tubers in 1997, but had no effect on infection from YNS or PNS in 1998 or 1999. The herbicides halosulfuron and pyrithiobac were evaluated in the greenhouse for efficacy against YNS and PNS +/- RKN. RKN did not affect herbicide efficacy. Both compounds reduced nutsedge root weights and total RKN numbers, but not RKN/gram root.

EVALUATION OF RHIZOSPHERE-COLONIZING ABILITIES OF FIVE POTENTIAL BACTERIAL BIOCONTROL AGENTS FOR *HETERODERA GLYCINES*. **Tian, Honglin, and R. D.** Department of Plant Pathology, University of Arkansas, Fayetteville, AR 72701.

Five chitinolytic bacterial isolates (C6, C10, C11, C31, C54) have suppressed reproduction of *H. glycines* in a silt loam soil amended with 0.4–0.6% (w/w) chitin. This research evaluated their ability to colonize the rhizosphere of 'Hutcheson' soybean over a 30-day period in soil with or without 0.4% chitin. One strain of each isolate that was rifampicin-resistant had a growth rate and chitinolytic ability similar to the wild strain. Bacteria were applied to soil around the roots of soybean seedlings at transplant. Total number of rifampicin-resistant cells in the rhizosphere and number of cells per gram dry root were determined at 5-day intervals. The bacterial strains exhibited different colonization patterns in soil with chitin, but colonization was low for all strains in soil without chitin. In soil with chitin, the total number of bacterial cells and the number/g dry root were 10 to 100 times more than in soil without chitin. The rifampicin-resistant C6 strain was the best colonizer. Total bacterial cells of this strain increased with time, and the number/g dry root was stable.

EXPRESSION OF NEMATODE RESISTANCE IN CULTIVATED PEANUT. **Timper, P., C. C. Holbrook, and H. Q. Xue.** USDA ARS, P. O. Box 748, Tifton, GA 31793.

Several cultivated peanut genotypes have moderate resistance to *Meloidogyne arenaria*. Our objective was to determine the expression of resistance for six of these genotypes. We examined four potential expressions: 1) reduced penetration, 2) fewer second-stage juveniles (J2) establish functional feeding sites, 3) slower maturation, and 4) reduced fecundity. Peanut seedlings were inoculated with J2 of *M. arenaria*, and transplanted 3 days later to synchronize nematode development. The resistant genotypes were compared to the cultivar Florunner (Flr), a susceptible control. The number of juveniles within the roots of resistant genotypes was similar to Flr after 3 days, but lower than Flr in two of the genotypes after 10 days. After 17 days, a greater percentage of J2 failed to develop in all of the resistant genotypes (72 to 79%) compared to Flr (50%). Of those that did begin to develop, the rate of maturation and fecundity was similar in Flr and the resistant genotypes. The primary expression of resistance in the six peanut genotypes appears to be a reduction in the percentage of J2 that initiate a functional feeding site and begin to develop. Egression from roots after penetration may be a consequence of not establishing a feeding site.

A RAPID MICRO-BIOASSAY FOR TESTING THE ANTAGONISTIC POTENTIAL OF FUNGI AGAINST THE BEET CYST NEMATODE. **Tischer, Tanja, X. Gao, and J. O. Becker.** Department of Nematology, University of California, Riverside, CA 92521.

Various species of fungi have been reported to be associated with biological suppression of *Heterodera schachtii*. It is desirable to determine the antagonistic potential of a fungal strain prior to more complex greenhouse or field trials. Our method utilized small glass vials (5-cm-length; 1-cm-diam.), filled with moist sandy soil which was infested with the test fungus as well as with 30 nematode cysts. After a 2, 3 or 4 week incubation, a pre-germinated radish seed was placed in each vial and allowed to grow for 5 days at 20 °C. The roots were recovered and stained with acid fuchsin for nematode enumeration. This technique is rapid, requires very little space, and allows tests for variations in soil physical properties and environmental conditions.

HETERODERA GLYCINES PATHOGENICITY AND FECUNDITY: DEVELOPING A GENETIC MODEL OF A PATHOGEN'S LIFE HISTORY TRAITS. **Tourjee, K. R., and T. L. Niblack.** Department of Plant Microbiology and Pathology, University of Missouri, Columbia, MO 65211.

A two-factor model for parasitism in natural populations of *Heterodera glycines* was confirmed based on race determination test results. These tests were done on nematodes extracted from soil samples obtained from 286 soybean fields throughout Missouri over a ten-year period. The test uses female counts from the differential host series including Pickett, Peking, Plant Introduction (PI) 88788, and PI 90673. Maximum likelihood confirmatory factor analysis was used to evaluate a model with two latent variables (factors 1 and 2) and 4 indicator variables (count data from each differential). Each observation was the average of three replicates. The standardized factor coefficients of factor 1 loaded most strongly on Pickett, Peking, and PI 90763. Factor 2 loaded negatively on Peking and PI 90763 but positively on PI 88788. Factor 1 had greater loadings than factor 2 for each differential. The squared multiple correlations for Pickett, Peking, and PI 90763 with the model were 0.77, 0.91, and 0.65, respectively; however, the squared multiple correlation for PI88788 was only 0.29, indicating that the two factors inadequately described the variability associated with this differential. This model was consistent with a discriminant analysis of data from *H. glycines* isolates maintained on the differentials for at least 20 generations. Hierarchical cluster analysis of discriminant scores revealed two main clusters. The model was further validated by ongoing selection experiments designed to isolate *H. glycines* genotypes homozygous for virulence genes. Selection experiments for fecundity (numbers of eggs per cyst) are also ongoing. Nonselected populations vary over a ten-fold range in their fecundity. The pathogenicity and fecundity results provide an opportunity to define more precisely the genetic variability of *H. glycines* life history traits.

THE FIRST REPORT OF THE CYST NEMATODE *CACTODERA* (NEMATODA: *HETERODERIDAE*) ON CEREALS IN CENTRAL HIGHLANDS OF MEXICO. **Tovar-Soto, A.,[1] I. Cid del Prado-Vera,[2] J. M. Nicol,[3] K. Evans,[4] J. S. Sandoval-Islas,[2] and A. Martínez-Garza.[5]** [1]ENCB-IPN. C.P. 11340, México, D.F. (becario COFAA)/IFIT-Colegio de Postgraduados. C.P. 56230, Montecillo, México, [2]IFIT-Colegio de Postgraduados, C.P. 56230, Montecillo, México, [3]International Maize and Wheat Improvement Centre (CIMMYT), México, [4]IACR Rothamsted Experimental Station, U.K., [5]ISEI-Colegio de Postgraduados, C.P. 56230, Montecillo, México.

During 1999, 86 cereal fields were sampled in 39 localities in the states of Hidalgo and Tlaxcala in central Mexico. Nematodes were extracted from soil using the Fenwick Can and from roots by hand dissection. Two major types of cysts were extracted: citriform and spherical. Morphometrics and morphology of 20 to 30 cysts, juveniles, females and eggs indicate the majority belong to genera Cactodera. We suggest our species is *Cactodera milleri*. However morphology and morphometrics of this population reveal differences with our species. This is the first report of *Cactodera* spp. on cereals in Mexico.

ASSESSMENT OF THE CONTAMINATION STATUS OF SOILS AND SEDIMENTS USING NEMATODE COMMUNITY ANALYSIS. **Trett, M. W., S. J. Forster, B. Calvo Urbano, S. P. Trett, J. D. Hutchinson, R. L. Feil and J. Green.** Physalia Ltd Consultant & Forensic Ecologists, Sedgefen House, 37 Meadow Walk, Harpenden AL5 5TF England.

Over the past 20 years, meiofauna and communities of free-living nematodes have provided the basis for the detection, delineation and monitoring of how industrial and domestic wastes impact aquatic environments at sites throughout the world. These studies capitalize on the abundance of nematodes, their species richness, the spectrum of sensitivities to different types of stress, and their comparatively short generation times. With increasing concern regarding the legacy of industrial contamination of terrestrial sites, the present paper considers the results of monitoring surveys that have used soil nematodes (U.K. and Australia) to assess the status of contaminated land sites. In a heavy metal contaminated site, ''hot-spots'' were detected and characterized by reduced abundances, lower species richness values, and increased dominance values. Correlation analyses identified species with differing responses to different soil metal concentrations. Results of preliminary studies of sites contaminated with organic chemicals are less clear. Problems associated with the application of this technique to terrestrial sites will be discussed.

EFFECTIVENESS OF SOIL ANALYSIS FOR PRESENCE OF THE SOYBEAN CYST NEMATODE, *HETERODERA GLYCINES*. **Tylka, G. L., and P. H. Flynn.** Department of Plant Pathology, Iowa State University, Ames, IA 50010-1020.

The soybean cyst nematode, *Heterodera glycines*, is a widespread but often undetected pathogen of soybean throughout the north central United States. Considerable effort is made to have fields in this region of the country sampled for presence of the nematode. Research was conducted in 1998 and 1999 to assess the effectiveness of soil analysis for presence of *H. glycines*. A wet-sieving and decanting technique was used to recover cysts from soil samples sent to the Iowa State University Plant Disease Clinic for testing for *H. glycines*. Sediments from 100 cm^3 soil per sample were recovered on a 250-μm-pore sieve. This material, containing *H. glycines* cysts, then was macerated with a motorized stainless steel pestle and poured through a 75-μm-pore sieve nested over a 25-μm-pore sieve. Materials caught on the 25-μm-pore sieve, which would retain nematode eggs, were stained with acid fuchsin, and *H. glycines* egg population densities were determined by microscopic observation. Susceptible, 7-day-old soybean seedlings were transplanted into soil remaining from nearly 800 samples with egg population densities of 0 to 500 eggs per 100 cm^3 soil. Seedlings were grown in the soil at 26 °C for 28 to 35 days, after which roots were observed macro- and microscopically for the presence of *H. glycines* females. The percentage of samples in which females were observed on soybean roots was compared among groups of samples representing a range of egg population densities. *H. glycines* females were observed on soybean roots grown in

14% of the samples in which no eggs were observed. The percentage of samples in which females formed on soybean roots increased from 48% for samples with 1 to 50 eggs per 100 cm^3 soil to 78% for samples with 100 to150 eggs per 100 cm^3 soil. Eighty to 91% of samples with egg population densities of 150 to 500 eggs per 100 cm^3 soil produced females on soybean roots. These results indicate the extent to which this combination of extraction techniques may falsely characterize soil samples as either *H. glycines*-infested or noninfested.

GENE *Mi-9* CONFERRING HEAT-STABLE RESISTANCE TO *MELOIDOGYNE* IN *LYCOPERSICON PERUVIANUM* IS A HOMOLOGUE OF GENE *MI-1*. **Veremis, J. C., I. Kaloshian, and P. A. Roberts.** Department of Nematology, University of California, Riverside, CA 92521.

The inheritance of heat-stable resistance from the northern wild tomato accession LA2157 was evaluated in segregating F_2 and F_3 progenies derived from a hybrid of LA2157 with the homozygous susceptible accession LA392. The F_2 progeny segregated 3:1 (R:S) to root-knot nematode biotypes indicating that resistance in LA2157 is conferred by a single dominant gene, *Mi-9*. The phenotypic classification of 300 individuals within the progenies as susceptible or resistant was used to determine the linkage of *Mi-9* with markers on chromosome 6. The RFLP-PCR marker REX-1 linked to the *Mi-1* gene was polymorphic between the above parentals and tightly linked to *Mi-9*. Fine mapping positioned *Mi-9* on the short arm of chromosome 6. Homology of the *Mi-9* locus from LA2157 and the *Mi-1* locus was also investigated with molecular markers. Using primers designed to amplify *Mi-1*, a DNA fragment was amplified that cosegregates with *Mi-9*. This novel nematode resistance locus in accession LA 2157, may be one of the ancestral stocks in the co-evolutionary development of the tomato complex. Genetic and molecular characterization of *Mi-9* will clarify its relationship to *Mi-1* and to the other novel heat-stable resistance. The overall goal is to understand the genetics, spectrum, and co-evolution of the resistance traits in order to incorporate the best characterized resistance into crops with classical and molecular methods.

THE PERSISTENCE OF NON-SYMBIOTIC BACTERIA IN *GALLERIA MELLONELLA* LARVAE INFECTED WITH THE SYMBIOTIC BACTERIA (*XENORHABDUS* SPP.) OF ENTOMOPATHOGENIC NEMATODES (*STEINERNEMA* SPP.). **Walsh, K., and J. Webster.** Centre for Environmental Biology, Department of Biological Sciences, Simon Fraser University, Burnaby, B.C.,Canada, V5A 1S6.

Insect cadavers infected with entomopathogenic nematodes (EPN's) do not putrefy despite the presence of the bacterial symbiont and other bacterial species. Experiments were done to determine the nature and source of the non-symbiotic bacteria and the antibiotic activity. Extracts (1.0 ml) from surface sterilized, macerated, larval cadavers of *Galleria mellonella*, infected with either *Steinernema feltiae* A21 strain (symbiont *Xenorhabdus nematophilus*) or *S. glaseri* (symbiont *X. poinarii*), were examined at successive time intervals up to 200 h post infection. Species diversity and population size of the bacteria differed depending on the species of EPN infecting the *G. mellonella* larvae. Larvae infected with *S. feltiae* contained low numbers of two non-symbiont species, one of these persisted and grew to a maximum population at about 140 h post infection in the presence of *X. nematophilus* while the other was eliminated by 30 h post infection. In vitro bioassays showed that metabolites from *X. nematophilus* were the probable cause of eliminating one of these bacteria but was only very weakly active against the other. This latter species showed very weak antibacterial activity against *X. nematophilus*. The bacterium that was eliminated by the antibiotic activity of *X. nematophilus* originated in the larval gut, and the persistent bacterium originated from within the EPN. *Galleria* infected with *S. glaseri* contained only *X. poinarii* and the bacterium that originated in the larval insect gut.

DETERMINE THE EFFECT OF *HETERODERA GLYCINES* ON SOYBEAN GROWTH, DEVELOPMENT, AND YIELD. **Wang, J.,[1] T. L. Niblack,[1] C. Marett,[2] O. Myers,[4] G. R. Noel,[3] C. Schmidt,[4] M. E. Schmidt,[4] J. Tremain,[1] G. L. Tylka,[2] and W. J. Wiebold.[1]** [1]Department of

Plant Pathology, University of Missouri, Columbia, MO 65211, [2]Department of Plant Pathology, Iowa State University, Ames, IA 50011, [3]Department of Crop Sciences, University of Illinois, Urbana, IL 61801, and [4]Department of Plant, Soil, and General Agriculture, Southern Illinois University, Carbondale, IL 62901.

Experiments were conducted at four locations naturally infested with *Heterodera glycines* in Iowa, Illinois, and Missouri from 1997 to 1999. A wide range of infestation levels occurred in relatively small fields at all locations. At each location, two locally adapted cultivars either resistant or susceptible to *H. glycines* were planted in 0.76-m-wide rows. For each cultivar, 20 1-m-long plots were sampled every 2 weeks starting 4 weeks after planting to assess plant growth and development. The sampled plots were randomly selected based on *H. glycines* population densities at planting so that a similar range of densities was sampled every 2 weeks. Plant growth and biomass accumulation of resistant and susceptible cultivars were similar. *Heterodera glycines* affected all yield components. Resistant cultivars consistently yielded better than susceptible cultivars under high nematode population densities. Numbers of seeds showed the least difference between cultivars among all yield components. *Heterodera glycines* can reduce yields without reducing other plant measurements. Damage threshold for susceptible varieties ranged from 400 to 5,000 eggs per 100 cm^3 soil at planting over all environments.

MECHANISMS OF *ROTYLENCHULUS RENIFORMIS* SUPPRESSION BY *CROTALARIA JUNCEA*. **Wang, Koon-Hui, and B. S. Sipes.** Department of Plant Pathology, University of Hawaii at Manoa, Honolulu, HI 96822.

Crotalaria juncea 'Tropic Sun' suppressed *Rotylenchulus reniformis* population densities in 3 pineapple-cover crop trials. The objective of this research was to determine the mechanisms involved in the suppression. Three experiments were conducted to elucidate (i) host status, (ii) allelopathic effect, and (iii) nematode-trapping fungal density enhancement ability of *C. juncea. C. juncea* was a poor host to *R. reniformis* (Rf = 1.37) as compared to pineapple (Rf = 10.86). *C. juncea* root leachate suppressed nematode egg hatch and percentage of mobile nematodes to 17% and 29% respectively as opposed to 93% and 84% in pineapple root leachate. When *R. reniformis* was incubated in the leaf leachate, 2% were mobile in *C. juncea* leaf leachate, but 85% were mobile in the pineapple leaf leachate. Nematode-trapping fungal population density from *C. juncea* treated plots was higher (43/g soil) than that in fallow with weeds followed by 1,3-D treatment (0 /g soil, $P<0.05$). Three proposed mechanisms are all involved in *R. reniformis* suppressiveness by *C. juncea.*

TWO PINEAPPLE-COVER CROP PLANTING SYSTEMS FOR *ROTYLENCHULUS RENIFORMIS* MANAGEMENT. **Wang, Koon-Hui, and B. S. Sipes.** Department of Plant Pathology, University of Hawaii at Manoa, Honolulu, HI 96822.

Crotalaria juncea 'Tropic Sun', *Brassica napus* 'Dwarf Essex' and *Tagetes erecta* 'Cracker Jack' were grown as intercycling cover crops between pineapple crops for 3 months, and intercropped with pineapple for 18 months. Nematode and nematode-trapping fungal population densities were monitored and compared to those in bare fallow, fallow with weeds, and 1,3-D treatments. In the intercycle system, *R. reniformis* numbers in cover crop-treated plots 6 months after pineapple planting did not differ from those in the 1,3-D treatment ($P>0.05$). The lowest rhizosphere *R. reniformis* populations and highest nematode-trapping fungal population densities ($P<0.05$) occurred in the *C. juncea* plots. In the intercrop system, *R. reniformis* populations and viability were lower in *C. juncea* and *B. napus* plots than in *T. erecta* and pineapple plots ($P<0.05$). Nematode-trapping fungal population densities were highest in *C. juncea* plots. Nematode suppressive effects of *C. juncea* became greater as the cropping period increased.

FLOW OF GENES FOR PARASITISM ON FOUR SOYBEAN DIFFERENTIALS FROM RACE 5 AND 14 TO RACE 3 OF *HETERODERA GLYCINES* BY IN-VIVO MASS CROSSES. **Wang,**

Shouhua, and R. D. Riggs. Department of Plant Pathology, University of Arkansas, Fayetteville, AR 72701.

An isolate of soybean cyst nematode race 3, to which the four soybean differentials (''Pickett'', ''Peking'', PI88788, and PI90763) were immune, was used as a maternal parent to cross with two single female isolates (SFI) of race 5 and one SFI of race 14. Race tests showed that SFIs of race 5 and 14 produced over 1,000 females on all positive differentials and near zero on all negative ones. In vivo mass crosses were made by placing 450 to 700 males of SFI's of race 5 or 14 around the root of 'Lee74' soybean that had been infected for 15 days with the race 3 isolate. After 30 days, females were screened and their eggs were used to infest the four differentials. In the crosses with two SFI's of race 5, up to 20 females/plant were produced on Pickett and PI88788. In the cross with race 14, the average number of females obtained was 90 on Pickett, 11 on Peking, and 10 on PI90763. The females obtained from Pickett were then placed on roots of Lee 74 and Pickett plants. The number of females on Pickett was 39% of the number of females on Lee74. These results indicate that fertilization by males of other races will change reproductive ability on differentials.

INADVERTANT SELECTION ALTERS INFECTIVITY, REPRODUCTION, AND LONGEVITY OF *HETERORHABDITIS BACTERIOPHORA*. **Wang, Xiaodong, and P. S. Grewal.** Entomology Department, Ohio State University, Ohio Agricultural Research and Development Center, Wooster, OH 44691.

Maintenance of genetic diversity is a critical issue in the successful application of biological control agents. We evaluated the effect of inadvertent selection during maintenance on the infectivity, fecundity, and longevity of infective juveniles (IJs) of *Heterorhabditis bacteriophora* GPS11 strain. The nematodes were maintained for 6 months in three different ways: LNL, IJs stored in liquid nitrogen for 6 months; 10L, IJs stored at 10 °C and cultured in *Galleria mellonella* every 2 months (i.e. three passages); RL, IJs maintained at room temperature and cultured in *G. mellonella* every two weeks (i.e. 12 passages). Both LNL and 10L IJs showed higher reproductive potential than RL IJs, whereas infectivity of the RL was higher than 10L and LNL. The survival of 10L and LNL was better than RL IJs after 14 weeks of storage at 25 °C. Results support the hypothesis that repeated subculturing of *H. bacteriophora* in *G. mellonella* reduces nematode fecundity and longevity, but increases infectivity.

DIRECT EXTRACTION AND ANALYSIS OF CYTOPLASMIC CONTENTS OF GIANT CELLS INDUCED IN HOST ROOTS BY *MELOIDOGYNE JAVANICA*. **Wang, Z.-H., R. H. Potter, and M. G. K. Jones**. Western Australian State Agricultural Biotechnology Centre, Murdoch University, Perth, Western Australia, 6150.

A modified pressure probe with a glass micropipette (5-10μm tip diameter) filled with silicone oil has been used to extract cytoplasmic contents directly from giant cell complexes in tomato roots infected with *Meloidogyne javanica*. The presence of multinucleate giant cell cytoplasm in the extracts has been confirmed by staining them with DNA fluorescing dyes and identification of nuclei by confocal laser scanning microscopy and epifluorescence microscopy. After combining 1 to 20 extracts of giant cell cytoplasm, and isolation of mRNA using oligo-dT magnetic beads, Differential Display RT-PCR has been carried out to compare gene transcripts expressed in giant cell cytoplasm and in comparable root segments. The same system has been used to measure levels of specific transcripts by semi-quantitative single cell RT-PCR (using real time fluorescence PCR) from giant cell cytoplasm at different times after infection. In both cases the levels of gene expression have been normalized with respect to actin and GAPDH (glyceraldehyde-3-phosphate dehydrogenase) expression. The system was tested by a analysis of transcripts previously identified in feeding cells. Two transcripts showed up-regulation (Lemmi 9 and IC9), and transcripts from the tomato RB7 gene were amplified specifically and yielded bands at the expected size. This system provides a direct method to probe giant cell function.

ADDITIONAL STUDIES UTILIZING SOYBEAN MEAL-BASED COMPOSITIONS AS ORGANIC AMENDMENTS FOR CONTROL OF *MELOIDOGYNE* SPP. **Weaver, C. F. and R. Rodríguez-Kábana.** Department of Plant Pathology, Auburn University, Alabama 36849-5409.

Two greenhouse experiments were conducted to assess the nematicidal activity of soybean meal (SBM) alone and in combination with either sorghum meal (SGM) or pearl millet meal (PMM). Soil for the experiments was heavily infested with a mixture of root-knot nematodes (RKN; *Meloidogyne incognita* + *M. arenaria*). The SBM was applied at rates of 0, 2.5 and 5.0 g/kg soil, and to each of these were added either SGM or PMM at rates of 0, 2.0, 4.0, and 8.0 g/kg soil. Nematode populations were assessed 10 days after treatment and again after 'Young' soybean was allowed to grow for 8 weeks. Results from the 10-day post-treatment sampling showed that SBM, SGM and PMM were all effective in suppressing RKN when used alone and that combination treatments did little to further enhance the suppressive effect over the short term. This was in contrast to the eight-week-sampling which did show a more synergistic effect in the combination treatments over the long-term, with SBM + SGM being more effective in suppressing RKN than was SBM + PMM.

ENTOMOPATHOGENIC SYMBIONTS: NEMATOLOGY'S KEY TO THE 21ST CENTURY. **Webster, John M.** Department of Biological Sciences, Simon Fraser University, Burnaby, Vancouver, BC, V5A 1S6.

Steinernematid and heterohabditid nematodes together with their bacterial symbionts, *Xenorhabdus* spp. and *Photorhabdus* spp., respectively, are becoming established biological control agents of some insect pests such as black vine weevil and citrus weevil. An informed, well-tested integration of these entomopathogenic nematodes into integrated pest management programs will consider their influence on the rhizospheric flora. The bacterial symbionts are protected and vectored by the dauer stage of the nematode in the soil and are consumed by the insect saprophytic stage of the nematode within the dead insect. The insect defense system is overcome within a few hours, and the multiplying bacterial symbionts in the insect cadaver produce secondary metabolites with a range of bioactivities. Some of these compounds are antimycotic (eg. xenocoumacins, nematophin), antibiotic (eg. xenorxides, indoles) and nematicidal (eg. stilbenes). These metabolites help diminish the number of competitors of the symbionts and may have other roles in the biology of their respective symbionts. Some of the smaller molecules can be synthesized and their bioactivities could be utilized by the pharmaceutical and agroforestry industries.

IMPACT OF ORGANIC MATTER MANAGEMENT ON PLANT-PARASITIC NEMATODES, THEIR DAMAGE TO HOST CROPS, AND SOIL HEALTH. **Widmer, T. L., and G. S. Abawi.** Dept. of Plant Pathology, NYSAES, Cornell Univ., Geneva, NY 14456.

Organic matter and its replenishment has become a major component of soil health management programs. Many of the soil's physical, chemical, and biological properties are a function of organic matter content and quality. Other symposium speakers will cover the numerous benefits of organic matter. This presentation will deal with the influence of various sources and forms of organic matter, specifically the use of cover crops as green manures, on nematode populations and resulting damage to host crops. The information will contribute to the design of nematode suppressive practices as a component of sustainable soil health management. More than 20 cover and rotational crops are being promoted for vegetable systems in New York. These were evaluated for their efficiency as nematode hosts and for suppression when incorporated as a green manure against the lesion and northern root-knot nematodes. The results obtained will be presented to illustrate the difference a crop selection can make on nematode populations, their damage to host crops, and thus soil health. Other various organic amendments also exhibited suppressive effects against these nematodes.

WHAT WE KNOW ABOUT HOW THE NEMATODE RESISTANCE GENE MI WORKS. **Williamson, V. M.,[1,2] C. F. Hwang,[2] G. Truesdell,[2] K. Fort,[2] and C. Branch.[1]** [1]Nematology Department and [2]CEPRAP, University of California, Davis, CA 95616.

The tomato Mi gene confers resistance against root-knot nematodes and potato aphids. Chimeric constructs between the functional gene, Mi-1.2, with a homologue, Mi-1.1, were produced, and their phenotypes examined in *Agrobacterium rhizogenes*-transformed roots. Some exchanges resulted in the loss of ability to confer nematode resistance whereas others resulted in a lethal phenotype. Transient expression of the latter class of chimeric constructs in *Nicotiana benthamiana* leaves produced localized cell death. The phenotypes of these constructs indicate that the LRR region of Mi-1.2 has a role in signaling localized cell death and that the N-terminal 161 amino acids have a role in regulating this death. Two additional approaches are being used to investigate the plant resistance response. Proteins that interact directly with the Mi product have been identified using the yeast two-hybrid system. Chemical inhibitors have been used to identify components of the Mi resistance pathway.

DIAGNOSTICS FOR DISTINGUISHING *MELOIDOGYNE CHITWOODI* AND *M. FALLAX* FROM OTHER ROOT KNOT NEMATODES. **Wishart, J., V. C. Blok, J. T. Jones, and M. S. Phillips.** Department of Mycology, Bacteriology and Nematology, Scottish Crop Research Institute, Invergowrie, Scotland DD2 5DA.

Meliodogyne chitwoodi and *M. fallax* have recently become quarantine organisms in the European community. *M chitwoodi* is thought to have been introduced into the Netherlands from the United States, probably by infested tubers, ornamental or root-stock material. These nematodes are serious pathogens of several European crops particularly potato, but also infect alfalfa, the cereals, maize, beet, carrot, and others. Because of this wide host range, nematodes are difficult to control. We have developed a sensitive PCR-based diagnostic which can be used to distinguish single juvenile or female nematodes of *M. chitwoodi* and *M. fallax* from other *Meloidogyne* spp. (*M. hapla, M. incognita, M. javanica, M. arenaria* and *M. mayaguensis*). We have also produced antisera to these nematodes which can be used detect infected plant material.

SPECIES IDENTIFICATION OF *XIPHINEMA* (NEMATODA: *LONGIDORIDAE*) OCCURRING IN ARKANSAS, USA BY MORPHOLOGICAL AND PCR-RFLP OF THE RIBOSOMAL DNA ITS REGION. **Ye, Weimin and R. T. Robbins.** Plant Pathology Department, University of Arkansas, Fayetteville, AR 72701.

From 1998 to 1999, 557 soil samples were taken from non-cultivated plants representing 25 counties in Arkansas. *Xiphinema americanum* Cobb, 1913, *X. bakeri* Williams, 1961, and *X. chambersi* Thorne, 1939 were found in numerous locations and were identified based on morphological and morphometric characteristics. The rDNA ITS region of these three species was amplified using a 18S primer (5′-TTGATTACGTCCCTGCCCTTT-3′) and a 26S primer (5′-TTTCACTCGCCGTTACTAAGG-3′). The pattern of restriction bands obtained with Alu, HinfI, DdeI, MspI and MboI clearly separate the three species. *Xiphinema krugi* Lordello, 1955 was found recently in a single location. Four other species, all in *Xiphinema americanum* group, namely *X. tenuicutus* Lamberti & Bleve-Zacheo, 1979, *X. rivesi* Dalmasso, 1969, *X. citricolum* Lamberti & Bleve-Zacheo, 1979 and *X. californicum* Lamberti & Bleve-Zacheo, 1979, had been reported in Arkansas in the literature.

SOYBEANS RESISTANT TO MULTIPLE *HETERODERA GLYCINES* POPULATIONS ATTACKING HARTWIG CULTIVAR. **Young, L. D.** USDA ARS, P. O. Box 345, Stoneville, MS 38776-0345.

Six *Heterodera glycines* populations were selected in the greenhouse from field populations for their ability to reproduce on Hartwig cultivar, a multiple-race-resistant soybean with resistance derived from plant introduction (PI) 437654. Planting histories of the fields were unknown except they were planted to soybean prior to sampling. These populations were compared in two green-

house tests with LY1 and LY2, populations previously reported to reproduce on Hartwig, for parasitism of Lee 68, Hartwig, and eight plant introductions resistant to either LY1 or LY2. The LY1 population resulted from selection within progenies of a race 2 by race 5 mass mating, whereas LY2 originated from field plots. Plant introduction 437655 was resistant to three of the populations in addition to LY2. Plant introduction 567516C was resistant to LY1 and two other populations. One population gave inconsistent results between two tests. Plant introductions 437655 and 567516C can be used in improved germplasm to control *Heterodera glycines* populations parasitizing PI 437654-derived cultivars if this becomes a problem.